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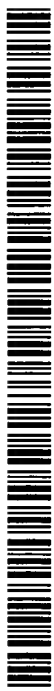
(54) Title: DIAGNOSIS AND TREATMENT OF BAFF-MEDIATED AUTOIMMUNE DISEASES AND CANCER

(57) Abstract: This invention relates to a method of determining a predisposition toward developing a cancer in a subject, particularly a subject selected from the group consisting of: (i) a subject having anti-TNF therapy or a candidate for anti-TNF therapy; (ii) a subject that is susceptible to an inflammatory disease or autoimmune disease, such as, for example, rheumatoid arthritis or colitis; and (iii) an aged subject comprising detecting elevated BAFF protein expression or elevated BAFF mRNA expression in a biological sample derived from the subject. In another embodiment, the present invention provides a method of detecting a cancer cell in a subject, particularly a subject selected from the group consisting of: (i) a subject having anti-TNF therapy or a candidate for anti-TNF therapy; (ii) a subject that is susceptible to an inflammatory disease or autoimmune disease, such as, for example, rheumatoid arthritis or colitis; and (iii) an aged subject, said method comprising detecting elevated BAFF protein expression or elevated BAFF mRNA expression in a biological sample derived from the subject. The invention also relates to a method of treating or preventing cancer in a subject selected from the group consisting of: (i) a subject having anti-TNF therapy or a candidate for anti-TNF therapy; (ii) a subject that is susceptible to an inflammatory disease or autoimmune disease, such as, for example, rheumatoid arthritis or colitis; and (iii) an aged subject comprising inhibiting or delaying the expression of BAFF mRNA or BAFF protein in the subject.

Diagnosis and treatment of BAFF-mediated autoimmune diseases and cancer

FIELD OF THE INVENTION

This invention relates to a method of determining a predisposition toward developing a
cancer in a subject, particularly a subject selected from the group consisting of: (i) a
subject having anti-TNF therapy or a candidate for anti-TNF therapy; (ii) a subject that is
susceptible to an inflammatory disease or autoimmune disease, such as, for example,
rheumatoid arthritis or colitis; and (iii) an aged subject comprising detecting elevated
BAFF protein expression or elevated BAFF mRNA expression in a biological sample
derived from the subject. In another embodiment, the present invention provides a
method of detecting a cancer cell in a subject, particularly a subject selected from the
group consisting of: (i) a subject having anti-TNF therapy or a candidate for anti-TNF
therapy; (ii) a subject that is susceptible to an inflammatory disease or autoimmune
disease, such as, for example, rheumatoid arthritis or colitis; and (iii) an aged subject,
said method comprising detecting elevated BAFF protein expression or elevated BAFF
mRNA expression in a biological sample derived from the subject. The invention also
relates to a method of treating or preventing cancer in a subject selected from the group
consisting of: (i) a subject having anti-TNF therapy or a candidate for anti-TNF therapy;
(ii) a subject that is susceptible to an inflammatory disease or autoimmune disease, such
as, for example, rheumatoid arthritis or colitis; (iii) an aged subject; and (iv) a subject
having a predisposition toward developing cancer eg., as determined using the diagnostic
method of the invention, said method comprising inhibiting or delaying the expression of
BAFF mRNA or BAFF protein in the subject.

BACKGROUND OF THE INVENTION

1. General

This specification contains nucleotide and amino acid sequence information prepared
using PatentIn Version 3.1, presented herein after the claims. Each nucleotide sequence
is identified in the sequence listing by the numeric indicator <210> followed by the
sequence identifier (e.g. <210>1, <210>2, <210>3, etc). The length and type of
sequence (DNA, protein (PRT), etc), and source organism for each nucleotide sequence,
are indicated by information provided in the numeric indicator fields <211>, <212> and
<213>, respectively. Nucleotide sequences referred to in the specification are defined by

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the term "SEQ ID NO:", followed by the sequence identifier (eg. SEQ ID NO: 1 refers to the sequence in the sequence listing designated as <400>1).

5 The designation of nucleotide residues referred to herein are those recommended by the IUPAC-IUB Biochemical Nomenclature Commission, wherein A represents Adenine, C represents Cytosine, G represents Guanine, T represents thymine, Y represents a pyrimidine residue, R represents a purine residue, M represents Adenine or Cytosine, K represents Guanine or Thymine, S represents Guanine or Cytosine, W represents Adenine or Thymine, H represents a nucleotide other than Guanine, B represents a 10 nucleotide other than Adenine, V represents a nucleotide other than Thymine, D represents a nucleotide other than Cytosine and N represents any nucleotide residue.

As used herein the term "derived from" shall be taken to indicate that a specified integer is obtained from a particular source albeit not necessarily directly from that source.
15

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of 20 matter.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers 25 but not the exclusion of any other step or element or integer or group of elements or integers.

Unless specifically stated otherwise, each feature described herein with reference to a particular aspect or embodiment of the invention shall be taken to apply *mutatis mutandis* 30 to each and every other aspect or embodiment of the invention. For example, any one or more features described herein with respect to methods for expression library construction shall apply to those embodiments relating to methods for screening expression libraries to identify a peptide or protein domain capable of binding a target protein or nucleic acid or nucleic acid encoding same.

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Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also
5   includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present invention is not to be limited in scope by the specific examples described
10   herein. Functionally equivalent products, compositions and methods are clearly within the scope of the invention, as described herein.

The present invention is performed without undue experimentation using, unless otherwise indicated, conventional techniques of molecular biology, microbiology, virology,
15   recombinant DNA technology, peptide synthesis in solution, solid phase peptide synthesis, and immunology. Such procedures are described, for example, in the following texts:

1.   Sambrook, Fritsch & Maniatis, , whole of Vols I, II, and III;

2.   DNA Cloning: A Practical Approach, Vols. I and II (D. N. Glover, ed., 1985), IRL
20   Press, Oxford, whole of text;

3.   Oligonucleotide Synthesis: A Practical Approach (M. J. Gait, ed., 1984) IRL Press, Oxford, whole of text, and particularly the papers therein by Gait, pp1-22; Atkinson *et al.,* pp35-81; Sproat *et al.,* pp 83-115; and Wu *et al.,* pp 135-151;

4.   Nucleic Acid Hybridization: A Practical Approach (B. D. Hames & S. J. Higgins,
25   eds., 1985) IRL Press, Oxford, whole of text;

5.   Animal Cell Culture: Practical Approach, Third Edition (John R.W. Masters, ed., 2000), ISBN 0199637970, whole of text;

6.   Immobilized Cells and Enzymes: A Practical Approach (1986) IRL Press, Oxford, whole of text;

30 7.   Perbal, B., A Practical Guide to Molecular Cloning (1984);

8.   Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.), whole of series;

9.   J.F. Ramalho Ortigão, "The Chemistry of Peptide Synthesis" *In:* Knowledge database of Access to Virtual Laboratory website (Interactiva, Germany);

- 4 -

10. Sakakibara, D., Teichman, J., Lien, E. Land Fenichel, R.L. (1976). *Biochem. Biophys. Res. Commun.* 73 336-342

11. Merrifield, R.B. (1963). *J. Am. Chem. Soc.* 85, 2149-2154.

12. Barany, G. and Merrifield, R.B. (1979) in *The Peptides* (Gross, E. and Meienhofer, J. eds.), vol. 2, pp. 1-284, Academic Press, New York.

13. Wünsch, E., ed. (1974) *Synthese von Peptiden in Houben-Weyls Metoden der Organischen Chemie* (Müler, E., ed.), vol. 15, 4th edn., Parts 1 and 2, Thieme, Stuttgart.

14. Bodanszky, M. (1984) *Principles of Peptide Synthesis,* Springer-Verlag, Heidelberg.

15. Bodanszky, M. & Bodanszky, A. (1984) *The Practice of Peptide Synthesis, Springer-Verlag,* Heidelberg.

16. Bodanszky, M. (1985) *Int. J. Peptide Protein Res.* 25, 449-474.

17. Handbook of Experimental Immunology, Vols. I-IV (D. M. Weir and C. C. Blackwell, eds., 1986, Blackwell Scientific Publications).

18. McPherson *et al., In: PCR A Practical Approach.,* IRL Press, Oxford University Press, Oxford, United Kingdom, 1991.

2. Description of the related art

In spite of numerous advances in medical research, cancer remains a leading cause of death throughout the developed world. Non-specific approaches to cancer management, such as surgery, radiotherapy and generalized chemotherapy, have been successful in the management of a selective group of circulating and slow-growing solid cancers.

Many solid tumors are considerably resistant to such approaches, and the prognosis in such cases is correspondingly grave. One example is brain cancer. Each year, approximately 15,000 cases of high grade astrocytomas are diagnosed in the United States. The number is growing in both pediatric and adult populations. Standard treatments include cytoreductive surgery followed by radiation therapy or chemotherapy. There is no cure, and virtually all patients ultimately succumb to recurrent or progressive disease. The overall survival for grade IV astrocytomas (glioblastoma multiforme) is poor, with about 50% of patients dying in the first year after diagnosis. Ovarian carcinoma is the fourth most frequent cause of female cancer death in the United States. Because of

its insidious onset and progression of ovarian cancer, about 65% to 75% of patients present with a tumor disseminated throughout the peritoneal cavity. Although many of these patients initially respond to the standard combination of surgery and cytotoxic chemotherapy, nearly 90 percent develop recurrence and inevitably succumb to their
5   disease.

Circulating cancers, such as, for example, leukemia, lymphoma and myeloma, originate in the bone marrow (in the case of leukemia and myeloma) or lymphatic tissues (in the case of lymphoma). Leukemia, lymphoma and myeloma are considered to be related cancers,
10   because they involve the uncontrolled growth of cells having similar functions and origins. The diseases may result from an acquired genetic injury to the DNA of a single cell, which becomes abnormal (malignant) and multiplies continuously. The accumulation of malignant cells interferes with the body's production of healthy blood cells and makes the body unable to protect itself against infections. Treatment of leukemia, lymphoma and
15   myeloma using chemotherapy and/or radiation therapy destroys malignant cells as well as healthy blood cells. Whilst allogeneic bone marrow transplantation (BMT) is an effective therapy useful in the treatment of many hematologic malignancies, it is often toxic to patients as a consequence of the patient developing raft-versus-host disease (GVHD). When a patient develops GVHD, treatment is successful only 50-75% of the
20   time; the remainder of the patients generally die. A related condition to GVHD is host-versus-graft disease (HVGD), or organ allograft rejection, which may occur, for example, when a donor intestine is transplanted into a patient with a diseased intestine. In this case, cells from the patient's immune system (the host) may attack the foreign intestinal tissue (the graft). While intestinal transplantation is not routine at the present time, such
25   techniques will likely become more common.

Because circulating and solid cancers are often difficult to detect at an early stage, and are generally aggressive and difficult to treat, new diagnostics and therapeutic treatments are needed.
30

The TNF family of proteins
Receptor ligands comprising the tumor-necrosis factor (TNF)-related cytokines are known to mediate host defense responses and regulate immune responses, particularly
35   inflammatory responses. Generally, proteins of the TNF family have fundamental

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regulatory roles in controlling the immune system and activating acute host defense systems.

The TNF family of ligands includes the proteins known in the art as (i) B cell Activation
5   Factor (BAFF); (ii) TNF; (iii) lymphotoxin-a (LT-a) ; (iv) LT-a/b; (v) FasL; (vi) CD40L; (vii) CD30L; (viii) CD27L; (ix) OX40L; and (x) 4-1BBL. Additonal TNF family proteins are described by Bodmer et al., TIBS 27, 19-26, 2002 which is incorporated herein by reference.

10   TNF and LTa are known to be secreted cytokines, in contrast with the other predominantly membrane anchored members of the TNF family. Secreted TNF may function as a general signal to cells that are distant from a stimulus that leads to receptor activation. TNF secretion may amplify an event leading to changes in the vasculature lining and the inflammatory state of cells.

15

In contrast, the membrane-bound members of the TNF family signal locally through TNF type receptors. For example, helper T cells provide CD40-mediated functions only to those B cells that are brought into direct contact with the T cell via a cognate T cell receptor (TCR) interaction. Similarly, cell-cell contacts limit the ability to induce cell death
20   (apoptsis) via the Fas system.

Certain TNF family proteins directly induce the apoptotic death of many transformed cells (Amakawa et al., Cell 84, 551-562, 1996; Nagata et al., Cell 88, 355-365, 1997; and Zheng et al., Nature 377, 348-351, 1995). Apoptosis is usually triggered following the
25   aggregation of death domains which reside on the cytoplasmic side of a TNF receptor, thereby activating the cysteine aspartate protein (caspase) pathway cascade (Nagata et al., Cell 88, 355-365, 1997). Some receptors for a TNF protein lack canonical death domains, however induce cell death weakly, such as, for example, LTb receptor and CD30. In fact, the known TNF family proteins are classified into three groups, based on
30   their ability to induce apoptosis, as follows:
1.   those that efficiently induce apoptosis in many cell lines (eg., TNF, FasL and TRAIL) such as, for example, in non-transformed lymphocytes (eg., FasL, and TNF);
2.   those that only weakly induce apoptosis (eg., TWEAK, CD30L and LTalb2), such

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as, for example, in non-transformed lymphocytes (eg., CD30L); and

3.   those that do not induce apoptosis (eg., CD40L).

The nucleotide sequences encoding these known TNF family proteins are only about 25%
to about 30% identical. The amino acid sequences of the corresponding proteins are at
least about 50% identical.

Notwithstanding the overall sequence divergence in the ligands, proteins in the TNF
family of ligands are characterized by a short N-terminal stretch of hydrophilic amino
acids, generally comprising several lysine or arginine residues thought to serve as stop
transfer sequences. This hydrophilic domain is followed by a transmembrane region and
an extracellular domain of variable length ("stalk") that separates a C-terminal receptor-
binding domain from the membrane. The C-terminal receptor-binding region comprises
the bulk of the protein, and may comprise glycosylation sites.

The structure of these proteins has been well-defined by crystallographic analyses of
TNF, LT-a, and CD40L. For example, TNF and LT-a are both structured into a sandwich
of two anti-parallel β-pleated sheets. Many of the amino acids that are conserved
between the different proteins occur in stretches within the scaffold β-pleated sheet. A
structural feature emerging from molecular studies of CD40L, TNF and LT-a is their
propensity to assemble into oligomeric complexes that appear to form the receptor
binding site at the junction between the neighboring sub-units, thereby creating a
multivalent ligand. The quaternary structures of TNF, CD40L and LT-a have been shown
to comprise trimers. It is likely that the basic sandwich structure is preserved in all of
these molecules, since portions of these scaffold sequences are conserved across the
various family members. The quaternary structure may also be maintained since the
subunit conformation is likely to remain similar.

Tumor necrosis factor (TNF)

TNF is a known mediator of septic shock and cachexia and is involved in the regulation of
hematopoietic cell development. It appears to play a major role as a mediator of defense
against bacteria, viruses and parasites, as well as having anti-tumor activity. TNF is also
involved in different autoimmune diseases. TNF is also a key mediator of the
inflammatory response of mammals.

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TNF is produced predominantly by activated monocytes and macrophages, however is also be produced by activated lymphocytes, neutrophils, mast cells, NK cells and endothelial cells.

5

Signalling through the TNF receptors TNFR1 (p75) and TNFR2 (p55), which also bind lymphotoxin, triggers the activation of a caspase cascade leading to apoptosis. Such signalling can also activate transcription factors that result in cell survival/proliferation and inflammatory responses.

10

TNF can exist either as a membrane bound form or as a soluble secreted cytokine.

TNF inhibitors are used as therapeutic reagents for the treatment of rheumatoid arthritis (Brown *et al.*, *Arthritis and rheumatism 46*, 3151-3158).

15

Anti-TNF therapies
Known anti-TNF therapies consist of proteins e.g., monoclonal antibodies or biological response modifiers that bind to TNF thereby preventing its inflammatory action, in particular Etanercept, Remicade and Humira.

20

Humira is a monoclonal antibody against human TNFα, supplied by Abbott Laboratories. Humira is known generically as adalimumab. All references herein to "Humira" shall be taken to include a reference to a generic drug equivalent thereof, including adalimumab. and all such references herein to "Humira" shall be taken to include a reference to such a

25 generic drug. The recommended dose of Humira for adult patients with rheumatoid arthritis (RA) is about 40 mg administered every other week as a subcutaneous (s.c.) injection.

Etanercept (trade-name Enbrel; Immunex Corp) is a genetically engineered biological

30 response modifier protein that binds human TNF, for treatment of patients with myasthenia gravis, Crohn's Disease, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, and active ankylosing spondylitis (AS), a chronic inflammatory disease affecting primarily the lower back and joints. All references herein to "etanercept" shall be taken to include a reference to a generic drug equivalent thereof, including Enbrel.

35 Etanercept is injected sub-cutaneously. The dosage for adults is generally about 25mg

twice weekly. The dosage for children aged 4 to 17 years is about 0.4mg/kg (maximum 25mg) twice weekly.

Remicade (trade-name infliximab; Centocor, Inc.) is a monoclonal antibody that specifically targets and binds irreversibly to TNF-α on the cell membrane and in the blood. All references herein to "remicade" shall be taken to include a reference to a generic drug equivalent thereof, including infliximab. Remicade is for the treatment of ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis, Crohn's disease in which fistulas form, or rheumatoid arthritis. The recommended dose of Remicade for the treatment of rheumatoid arthritis is about 3 mg/kg body weight, given as an intravenous injection or infusion ,followed with additional similar doses at 2 and 6 weeks after the first dose is administered, then every 8 weeks thereafter. Remicade is prescribed for administration in combination with methotrexate. For patients who have an incomplete response, consideration may be given to adjusting the dose up to about 10 mg/kg or treating as often as every 4 weeks. The recommended dose of Remicade for treatment of Crohn's Disease is about 5 mg/kg given as an induction regimen at 0, 2 and 6 weeks, followed by a maintenance regimen of about 5 mg/kg every 8 weeks thereafter for the treatment of moderately-to-severely-active Crohn's disease, or fistulizing disease.

In a recent survey, Brown *et al.*, 2002, *Arthritis Rheum. 46: 3151*, identified 26 cases of lymphoproliferative disorders following treatment with etanercept (18 cases) or infliximab (8 cases), the majority of which (81%) were non-Hodgkin's lymphomas. The interval between initiation of therapy with etanercept or infliximab and the development of lymphoma was very short (median 8 weeks). In 2 instances (1 infliximab, 1 etanercept), lymphoma regression was observed following discontinuation of anti-TNF treatment, in the absence of specific cytotoxic therapy directed toward the lymphoma.

Factors that may contribute to this increased risk of lymphoproliferative malignancies in patients receiving anti-TNF therapy include use of immunosuppressive drugs (e.g., methotrexate, azathioprine and corticosteroids), infection with Epstein-Barr virus (EB virus), disease activity, widespread joint involvement, advanced age, poor functional class, and duration of the inflammatory disease.

B Cell Activation Factor (BAFF)

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B cell Activation Factor (BAFF) is a member of the TNF Family of ligands that is a survival factor for B cells and is necessary for B cell maturation in the periphery. Comparison of the BAFF sequence with other members of the human TNF family reveals considerable structural similarity, particularly in the extracellular domain. The nucleotide sequences encoding human and murine BAFF proteins and the derived amino acid sequences of the proteins *per se* are disclosed by Schneider *et al.*, *J. Exp. Med.* *189*, 1747-1756, 1999.

BAFF is present primarily in the spleen and in peripheral blood lymphocytes, strongly indicating a regulatory role in the immune system. BAFF is produced by neutrophils, macrophages and dendritic cells and has three known receptors. Two of the receptors, BCMA and TACI, are shared with a closely a related ligand called APRIL. The third receptor, BAFF-R appears to recognise only BAFF. All three receptors are expressed on B cells. TACI is also expressed by a subset of T cells.

Elevated levels of BAFF have been found in the serum of some patients with rheumatoid arthritis (RA) (approximately 19-25%), SS (36%) and SLE (23%). Aberrant expression of BAFF and APRIL have also been associated with B-cell chronic lymphocytic leukemia (B-CLL).

The generation of mice expressing murine BAFF protein is described in detail in WO 99/12964 (Biogen, Inc.). In that study, a full length murine BAFF protein was expressed in transgenic mice operably under the control of a liver-specific alpha-1 antitrypsin promoter having an APO E enhancer. Founder mice having a BAFF transgene introduced into their genomes either died at a young age, possibly as a consequence of cardiovascular and renal abnormalities, or alternatively, they exhibited proteinuria, and/or an abnormally elevated ratio of B220 positive B cells to CD4 positive T cells suggesting enhanced B cell numbers. In addition to B cell hyperplasia, BAFF transgenic mice displayed severe hyperglobulinemia associated with rheumatoid factor (RF) and circulating immune complexes (CIC). Analysis of secondary lymphoid organs in BAFF transgenic mice was also consistent with the expanded B cell phenotype showing multiple cellular abnormalities and intense immune activity. BAFF transgenic mice also exhibited symptoms similar to human Systemic Lupus Erythematosis (SLE) and Sjögrens Syndrome (SS) in that study. Local over expression of BAFF was shown to not induce any immunological or pathological events in those studies.

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It is also known that BAFF-deficient mice do not have mature B cells.

WO 99/12946 (Biogen, Inc.) also showed that soluble BAFF protein was present in the
blood of BAFF transgenic mice. Serum from BAFF transgenic mice was shown to block
the binding of mammalian cell-derived mouse soluble Flag-tagged BAFF to BJAB cells.

SUMMARY OF THE INVENTION
In work leading up to the present invention, the inventors sought to develop improved
diagnostics and therapeutics for inflammatory diseases and/or autoimmune diseases
such as, for example, rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's
disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active
ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel
disease arthritis, colitis or cancer.

The inventors noted that some rheumatoid arthritis (RA) patients have elevated levels of
BAFF protein. To determine whether or not BAFF protein was likely to participate in the
development of RA, the inventors tested the efficacy of anti-TNF treatments on blocking
certain phenotypes associated with BAFF expression. The inventors produced a TNF-
deficient mouse that ectopically expresses a BAFF protein (ie., $TNF^{-/-}$; $BAFF^{+/+}$) and
examined the phenotype of the mice as they aged. The inventors found that BAFF-
mediated autoimmune disease is not TNF-dependent, because the onset of autoimmune
symptoms is accelerated in $TNF^{-/-}$; $BAFF^{+/+}$ mice, and there is a high incidence of
hyperplasic lymphoid masses in such transgenic animals. However, the inventors
concluded that TNF probably has a protective effect against the BAFF-mediated risk of
lymphoma, since animals that over express BAFF but are not TNF nulls have a reduced
incidence of the disease compared to $TNF^{-/-}$; $BAFF^{+/+}$ animals. The data obtained by the
inventors suggested that patients having elevated serum BAFF, eg., aged or aging
members of the population at risk of developing RA, have an elevated risk of developing
adverse neoplastic diseases, particularly those neoplasms associated with anti-TNF
therapy.

Accordingly, one aspect of the present invention provides nucleic acid-based methods
and protein-based methods for diagnosing cancer in a human or other mammal.

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In one embodiment, there is provided a method for determining a cancer cell in a subject that has commenced or undergone anti-TNF therapy or a predisposition to developing cancer in a subject that is a candidate for anti-TNF therapy or has commenced or undergone anti-TNF therapy , said method comprising determining the level of expression of a BAFF gene in a sample of said subject wherein elevated expression of said gene relative to the level of expression of a BAFF gene in a normal or healthy subject that does not have elevated BAFF expression is indicative of said cancer cell or said predisposition to developing cancer and wherein said BAFF gene comprises a nucleotide sequence selected from the group consisting of:

(a)   a sequence encoding a polypeptide comprising an amino acid sequence having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4;

(b)   a sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3;

(c)   a sequence that hybridizes specifically under at least low stringency conditions to SEQ ID NO: 1 or SEQ ID NO: 3;

(d)   a sequence comprising a protein-encoding region of (b) or (c); and

(e)   a sequence complementary to any one of (a) to (d).

Preferably, the cancer is a lymphoma such as a non-Hodgkin's lymphoma (NHL), for example an NHL selected from the group consisting of diffuse large B cell NHL, large cell NHL, small T cell NHL, diffuse large cell NHL, B cell NHL, follicular mixed small and large cell NHL, mantle cell B cell NHL and small lymphocytic B cell NHL. In an alternative embodiment, the cancer is a leukemia or a myeloma or a carcinoma (e.g., a head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer, renal cell cancer, bladder cancer, a gynecological carcinoma, prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, epithelial vulval carcinoma or a medulloblastoma).

The sample may be any biological sample comprising a BAFF protein or mRNA encoding same subject to the proviso that for nucleic acid-based assays the sample must comprise cells that express a BAFF-encoding mRNA. Preferred samples for diagnostic assays of the present invention comprise blood or a fraction of blood such as, for example, serum, a fraction of serum preferably serum cleared of IgG, plasma, a fraction of plasma such as a buffy coat preparation or a fraction comprising a cell type selected from the group consisting of peripheral blood lymphocytes, neutrophils, macrophages, T cells and dendritic cells. Other samples, such as tissues expressing BAFF, are not to be excluded.

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In a preferred embodiment, the diagnostic method of the invention further comprises obtaining the sample from a subject.

5   Alternatively, or in addition, the diagnostic method of the invention further comprises processing a sample from the subject to produce a derivative or extract that comprises a BAFF protein or mRNA encoding a BAFF protein. Such extracts may, for example, additionally comprise a TNF protein or mRNA encoding a TNF protein.

10 In the assay formats described herein, the sample is prepared on a solid matrix such as for high throughput formats, or alternatively, the sample is solubilized.

The present invention clearly encompasses the use of assay samples that have been obtained previously from a subject.

15

In one embodiment, the expression of a BAFF gene is determined by a process comprising determining the level of a polypeptide encoded by the BAFF gene in a test sample from the subject. In accordance with this embodiment, it is preferred for the assay to comprise:

20 (a)   determining the level of a polypeptide encoded by the BAFF gene in a test sample from the subject; and

(b)   comparing the level of the polypeptide determined at (a) to the level of said polypeptide in a comparable sample from a healthy or normal subject that does not have elevated BAFF expression, wherein a level of a polypeptide at (a) that is enhanced in the

25 test sample relative to the comparable sample from the healthy or normal subject is indicative of elevated expression of a BAFF gene.

Preferably, the level of the polypeptide is determined by a process comprising contacting an antibody that binds specifically to a polypeptide encoded by the BAFF gene to the test

30 sample for a time and under conditions sufficient for an antigen-antibody complex to form and then detecting the complex.

Preferred immunoassay formats used in accordance with this embodiment comprise an immunohistochemical (IHC) detection means, an enzyme-linked immunosorbent assay

35 (ELISA), or a Western blot immunoassay, amongst others known to the skilled artisan.

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Antibodies that bind to a BAFF protein are preferably either polyclonal antibodies or monoclonal antibodies. Other antibody types are not excluded from use in the present context.

5   In alternative embodiment, the expression of a BAFF gene is determined by a process comprising determining the level of mRNA encoded by a BAFF gene in a test sample from the subject. In accordance with this embodiment, it is preferred for the method to comprise:

(a)   determining the level of mRNA encoded by a BAFF gene in a test sample from the
10   subject; and

(b)   comparing the level of mRNA determined at (a) to the level of mRNA encoded by a BAFF gene in a comparable sample from a healthy or normal subject that does not have elevated BAFF expression, wherein a level of mRNA at (a) that is enhanced in the test sample relative to the comparable sample from a healthy or normal subject is indicative of
15   elevated expression of a BAFF gene.

Preferably, the mRNA is detected by contacting a nucleic acid probe to nucleic acid in the test sample for a time and under conditions sufficient for hybridization to occur and then detecting the hybridization.

20

Preferred probes for this purpose comprise a nucleotide sequence selected from the group consisting of:

(a)   a sequence encoding an amino acid sequence having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4;
25   (b)   a sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3;

(c)   a sequence that hybridizes specifically under at least low stringency conditions to SEQ ID NO: 1 or SEQ ID NO: 3;

(d)   a sequence comprising a protein-encoding region of (b) or (c);

(e)   a sequence complementary to any one of (a) to (d); and
30   (f)   a sequence comprising at least about 20 contiguous nucleotides of any one of (a) to (e).

More preferably, the nucleic acid probe comprises a nucleotide sequence selected from the group consisting of:

(a)   a sequence encoding the amino acid sequence set forth in SEQ ID NO: 2;
35   (b)   the sequence set forth in SEQ ID NO: 1;

(c)   a sequence that hybridizes specifically under at least low stringency conditions to

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SEQ ID NO: 1;

(d)   a sequence comprising a protein-encoding region of (b) or (c);

(e)   a sequence complementary to any one of (a) to (d); and

(f)   a sequence selected from the group consisting of SEQ ID Nos: 9-140.

Still more preferably, the nucleic acid probe comprises a nucleotide sequence selected from the group consisting of SEQ ID Nos: 9-140.

As will be known to the skilled artisan, a nucleic acid probe can be labeled with a reporter molecule and hybridization is detected by detecting the reporter molecule. Alternatively, hybridization is detected by detecting nucleic acid amplified in a polymerase chain reaction (PCR).

The hybridization is carried out *in situ* on a test sample consisting of a histology specimen, or on a nucleic acid microarray of test samples or a tissue microarray of test samples or in solution.

The diagnostic assay of the present invention is useful for diagnosing cancer or a predisposition thereto in any mammalian animal or a human subject. As will be apparent from the disclosure herein of the effect of anti-TNF therapy and immunosuppressant drugs on the incidence of cancer in autoimmune patients, it is especially preferred that the subject has undergone or commenced anti-TNF therapy, preferably in combination with an immunosuppressive drug e.g., an immunosuppressive drug selected from the group consisting of methotrexate, azathioprine and a corticosteroid. Immune compromised subjects, especially those that are candidates for anti-TNF therapy, such as those infected with Epstein-Barr virus (EBV) or aged subjects, may also have a higher incidence of cancer or predisposition thereto when placed on anti-TNF therapy. It will also be apparent from the disclosure herein that the present invention is particularly useful for diagnosing a cancer and/or predicting a predisposition for cancer is a subject suffering from an autoimmune disease or inflammatory disease, preferably rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis or colitis.

In a preferred embodiment, the diagnostic assay further comprises determining the level of expression of a TNF protein or nucleic acid encoding a TNF protein in a biological

sample derived from the subject, wherein a reduced level of the TNF protein or nucleic acid encoding the TNF protein in the sample is indicative of a cancer cell in the subject or a predisposition for the cancer.

5   The present invention further encompasses any synthetic or recombinant peptides, or antibodies suitable for use in the assays described herein. Antibodies or fragments thereof are useful in therapeutic, diagnostic and research applications, including the purification and study of the receptor proteins, identification of cells expressing surface receptor, and sorting or counting of cells. Thus, the present invention encompasses the
10   use of an antibody or fragment thereof described herein (e.g., monoclonal antibodies or an antigen-binding fragment thereof) in therapy, including prophylaxis, or diagnosis, and use of such antibodies or fragments for the manufacture of a medicament for use in treatment of diseases or conditions as described herein.

15   A second aspect of the present invention provides for the use of an antibody that binds to a BAFF protein to detect a cancer cell in a sample from a subject that has commenced or undergone anti-TNF therapy or to detect a predisposition to developing cancer in a subject that is a candidate for anti-TNF therapy or has commenced or undergone anti-TNF therapy, wherein the BAFF protein comprises an amino acid sequence having at
20   least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4.

A further aspect of the present invention provides for the use of a nucleic acid probe that hybridizes specifically to mRNA encoding a BAFF protein to detect a cancer cell in a sample from a subject that has commenced or undergone anti-TNF therapy or to detect a
25   predisposition to developing cancer in a subject that is a candidate for anti-TNF therapy or has commenced or undergone anti-TNF therapy, wherein the mRNA encoding a BAFF protein comprises a nucleotide sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3. Preferred probes for this purpose comprise a sequence that is complementary to at least about 20 contiguous nucleotides of SEQ ID NO: 1 or SEQ ID
30   NO: 3 or alternatively, a nucleotide sequence set forth in any one of SEQ ID Nos: 9-140, especially an antisense sequence from amongst SEQ ID Nos: 9-140.

Also encompassed by the present invention are methods of identifying ligands of the BAFF polypeptide, such as, for example, inhibitors or antagonists, or alternatively,
35   agonists of BAFF function. In one embodiment, suitable host cells that have been

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engineered to express BAFF or a BAFF homolog encoded by nucleic acid having at least about 80% identity to SEQ ID NO: 1 are used in an assay to identify and assess the efficacy of ligands, agonists or antagonists of BAFF function. Such cells are also useful in assessing the function of the expressed BAFF protein or homolog. According to the
5   present invention, ligands, agonists, or antagonists of BAFF function are identified in a suitable assay, and further assessed for their therapeutic efficacy. Antagonists of BAFF are used to inhibit (ie. reduce or diminish or prevent) BAFF-mediated effects in cells, such as, for example, any hyperproliferative disease, inflammatory state, inflammation, or cancer.   Alternatively, ligands and/or agonists of BAFF are useful for inducing or
10 enhancing BAFF-mediated effects in cells, particularly in aging patients or as an adjunct to anti-TNF therapy.

Accordingly, a further aspect of the present invention provides a method of identifying a compound that reduces or antagonizes expression of a BAFF gene comprising:
15 (a)   administering a candidate compound to a cell that expresses a BAFF gene at an elevated level; and
(b)   determining the level of expression of a BAFF gene in the presence of the compound relative to the level of expression of the gene in the absence of the compound, wherein reduced level of expression of a BAFF gene in the presence of the compound
20 indicates that the compound is an antagonist of BAFF gene expression and wherein said BAFF gene comprises a nucleotide sequence selected from the group consisting of:
(i)   a sequence encoding a polypeptide comprising an amino acid sequence having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4;
(ii)   a sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3;
25 (iii)   a sequence that hybridizes specifically under at least low stringency conditions to SEQ ID NO: 1 or SEQ ID NO: 3;
(iv)   a sequence comprising a protein-encoding region of (ii) or (iii); and
(v)   a sequence complementary to any one of (i) to (iv).

30 Preferred cells for use in the screening assays over express a BAFF gene by virtue of having been stably transformed or transiently transfected with a nucleic acid comprising a BAFF gene. For example, 293 cells that have been stably transformed or transiently transfected with a nucleic acid comprising a BAFF gene are particularly suitable for this purpose. This aspect of the present invention clearly encompasses a preferred form
35 comprising obtaining or producing the transformed or transfected cell.

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Any class of candidate antagonist described herein can be tested in the inventive screening assay. Preferably, the test compound comprises siRNA or shRNA comprising a nucleotide sequence set forth in any one of SEQ ID Nos: 9-140. In an alternative
5   embodiment, the test compound comprises antisense RNA. In an alternative embodiment, the test compound comprises an antibody that binds to a BAFF protein.

In performing the screening assays of the invention to identify modulatory compounds, it is preferred for the level of BAFF gene expression to be determined by a process
10   comprising determining the level of a polypeptide encoded by the gene in the presence of the compound relative to the level of the polypeptide in the absence of the compound, wherein a reduced level of the polypeptide in the presence of the compound indicates that the compound is an antagonist of expression of the gene.

15   Preferably, the level of the polypeptide is determined by a process comprising:
      (a)   contacting the cell or a protein extract thereof with monoclonal or polyclonal antibody that binds specifically to protein encoded by the BAFF gene under conditions sufficient for an antigen-antibody complex to form; and
      (b)   detecting the antibody bound.
20

As will be apparent to the skilled artisan, preferred antibodies for performing this embodiment are similar in structure to those used in the diagnostic assay formats of the invention. Similarly, the methods for detecting antibody binding are similar to those used for detecting antibody binding to patient samples in diagnostic assay platforms.
25

In a particularly preferred embodiment, the screening assay comprises:
      (a)   providing a cell that expresses the polypeptide;
      (b)   incubating the cell in the presence and absence of a compound to be tested;
      (c)   contacting an extract of the cell comprising the polypeptide with the antibody under
30   conditions sufficient for an antigen-antibody complex to form thereby capturing the polypeptide; and
      (d)   detecting the antibody bound at (c).

In an alternative embodiment, the screening assay comprises:
35   (a)   providing a cell expresses the polypeptide;
      (b)   incubating the cell in the presence and absence of a compound to be tested;

(c)   contacting an extract of the cell comprising the polypeptide with the antibody under conditions sufficient for an antigen-antibody complex to form thereby capturing the polypeptide;

(d)   contacting the captured polypeptide with an antibody that binds to the polypeptide under conditions sufficient for an antigen-antibody complex to form, wherein said antibody binds to a different epitope on the polypeptide to the antibody at (c); and

(e)   detecting the antibody bound at (d).

Alternatively, in performing the screening assays of the invention to identify modulatory compounds, it is preferred for the level of BAFF gene expression to be determined by a process comprising determining the level of an mRNA transcription product of the gene in the presence of the compound relative to the level of the mRNA in the absence of the compound, wherein a reduced level of the mRNA in the presence of the compound indicates that the compound is an antagonist of expression of the gene. Preferably, the mRNA is detected by contacting a nucleic acid probe with the mRNA transcription product in the cell or an extract thereof for a time and under conditions sufficient for hybridization to occur and then detecting the hybridization.

As will be apparent to the skilled artisan, preferred nucleic acid probes for performing this embodiment are similar in structure to those used in the diagnostic assay formats of the invention. Similarly, the methods for detecting hybridization of such probes are similar to those used for detecting hybridization to patient samples in diagnostic assay platforms.

In a particularly preferred embodiment, the screening assay comprises:

(a)   performing the method of claim 79 to thereby identify or determine a compound that reduces or antagonizes expression of a BAFF gene;

(b)   optionally, determining the structure of the compound; and

(c)   providing the compound or modulator or the name or structure of the compound.

In an alternative embodiment, the screening assay comprises:

(a)   performing the method of claim 79 to thereby identify or determine a compound that reduces or antagonizes expression of a BAFF gene;

(b)   optionally, determining the structure of the compound;

(c)   optionally, providing the name or structure of the compound; and

(d)   producing or synthesizing the compound.

A further aspect of the present invention provides an isolated nucleic acid that

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antagonizes expression of a BAFF gene consisting of a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule, preferably comprising a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

5   A further aspect of the present invention provides a vector comprising an isolated nucleic acid that antagonizes expression of a BAFF gene consisting of a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule, preferably comprising a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

10   A further aspect of the present invention provides a cell comprising a vector that comprises an isolated nucleic acid that antagonizes expression of a BAFF gene consisting of a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule, preferably comprising a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

15

The present inventors have shown that TNF provides a protective effect against the development of a BAFF-mediated disease, such as, for example, a lymphoma. Accordingly, a further aspect of the present invention provides a prophylactic method of preventing or delaying the development of a BAFF-mediated disease in a subject

20   comprising administering an amount of an agent sufficient to agonize TNF or enhance the level of TNF protein or TNF activity in a cell of the subject.

The present invention clearly contemplates prophylactic and/or therapeutic treatments wherein the level of BAFF expression is reduced and/or the level of TNF is enhanced in a

25   subject in need thereof.

Accordingly, a further aspect of the present invention provides a method of treating a hyperproliferative disease, such as, for example, cancer, hyperimmune response, inflammatory or autoimmune disorder (eg. rheumatoid arthritis or colitis), autoimmune

30   disease, or graft rejection, comprising administering an antagonist of BAFF function to an individual (e.g., a mammal) for a time and under conditions sufficient to reduce or prevent BAFF activity in said individual, thereby reducing or preventing one or more BAFF-mediated effects will be known to those skilled in the art, the expression can be reduced at the RNA level or the protein level. .

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By "BAFF activity" is meant any activity of a BAFF protein including a function mediated by binding of BAFF to its receptor and any signalling effected thereby.

Preferably, the method comprises administering an amount of an agent that reduces
5   BAFF expression to the subject sufficient to reduce the level of a BAFF protein or mRNA encoding a BAFF protein in a cell of the subject. Accordingly, the antagonist may comprise nucleic acid, such as, for example, antisense nucleic acid, a ribozyme, or nucleic acid that forms a triple helical structure, capable of reducing BAFF expression in a cell of the individual. Antibodies that bind BAFF and inhibit its activity are also useful in
10   this context. As with the other embodiments described herein, it is particularly preferred to use an agent that reduces BAFF expression comprising a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule, or alternatively, an antibody that binds to a BAFF protein thereby inhibiting its activity. Particularly preferred antagonists for performing this embodiment comprise siRNA or shRNA comprising a nucleotide
15   sequence selected from the group consisting of SEQ ID Nos: 9-140. Such agents are able to be administered by injection or preferably, by non-invasive routes such as by intranasal inhalation. Other antagonists, e.g., dominant negative mutants, small molecules etc are not to be excluded. Preferably, the therapeutic/prophylactic method further comprises administering an amount of an agent sufficient to agonize TNF or
20   enhance the level of TNF protein or TNF activity in a cell of the subject. Preferred agents that agonize TNF or enhance the level of TNF protein or TNF activity are co-administered with an agent that reduces BAFF expression in the subject such that the level of BAFF and the level of TNF are modulated simultaneously in the subject.

25   The therapeutic/prophylactic method is suitable for treating any cancer, preferably a lymphoma, more preferably a non-Hodgkin's lymphoma (NHL) e.g., a NHL is selected from the group consisting of diffuse large B cell NHL, large cell NHL, small T cell NHL, diffuse large cell NHL, B cell NHL, follicular mixed small and large cell NHL, mantle cell B cell NHL and small lymphocytic B cell NHL. The treatment of a leukemia, a myeloma, or
30   a carcinoma e.g., a head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer, renal cell cancer, bladder cancer, a gynecological carcinoma, prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, epithelial vulval carcinoma or medulloblastoma, is also encompassed by the inventive method.
35

The therapeutic/prophylactic method is also suitable for the treatment of any mammal,

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especially a human.

In a preferred embodiment, the method prevents or delays the development of a cancer in a subject that is a candidate for anti-TNF therapy or has undergone or commenced
5   anti-TNF therapy. Preferred subjects for which the therapeutic method is particularly applicable are those subjects that have undergone or commenced anti-TNF therapy in combination with an immunosuppressive drug and have elevated BAFF expression, particularly in their sera. As will be known to a skilled clinician in the field of treatment of autoimmune diseases or inflammatory diseases, the immunosuppressive drug is
10 preferably methotrexate, azathioprine or a corticosteroid. Other subjects for which the therapeutic method is particularly applicable are those subjects infected with Epstein-Barr virus (EBV), or suffering from an autoimmune disease or inflammatory disease, or aged subjects.

15 A further aspect of the present invention provides for the use of a composition that reduces or inhibits BAFF expression or activity in combination with an agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) for treating an inflammatory disease or autoimmune disease in a subject. Preferably, the agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) is an antibody or polypeptide
20 that binds to TNFα thereby reducing or inhibiting the activity of TNFα. Such agents are known to those skilled in the field of anti-TNF therapies. It will also be known to the skilled artisan that an agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) can be used in combination with an immunosuppressant drug e.g., methotrexate, azathioprine or a corticosteroid.
25

A further aspect of the present invention provides for the use of a composition that reduces or inhibits BAFF expression or activity in combination with an agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) in the preparation of one or more medicaments for treating an inflammatory disease or autoimmune disease in a
30 subject.

A further aspect of the present invention provides for the use of an agent that reduces or inhibits BAFF expression or activity for treating an inflammatory disease or autoimmune disease in a subject wherein the subject is: (i) a candidate for anti-TNF therapy or has
35 undergone or commenced anti-TNF therapy; and (ii) has an enhanced BAFF activity or expression.

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A further aspect of the present invention provides for the use of an agent that reduces or inhibits BAFF expression or activity in the preparation of a medicament for treating an inflammatory disease or autoimmune disease in a subject wherein the subject is: (i) a

5   candidate for anti-TNF therapy or has undergone or commenced anti-TNF therapy; and (ii) has an enhanced BAFF activity or expression.

A further aspect of the present invention provides a commercial package comprising:

(a)   a composition that reduces or inhibits BAFF expression or activity in a subject;

10 (b)   an agent that reduces or inhibits TNFα activity; and

(c)   instructions for use of the composition and agent for treating an inflammatory disease or autoimmune disease in a subject.

Preferably, the composition that reduces or inhibits BAFF expression comprises a small

15 interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule or an antibody that binds to BAFF protein.

Preferred agents that reduce or inhibit TNFα are those compositions that are used in anti-TNF therapies, e.g., an antibody or polypeptide that binds to TNFα thereby reducing or

20 inhibiting the activity of TNFα.

As used herein, the term "anti-TNF therapy" shall be taken to mean any chemical, biochemical, pharmaceutical or other means that reduces the level or activity of tumor necrosis factor (TNF), especially tumor necrosis factor-alpha (TNF-α) in a subject in need

25 thereof, such as, for example, a subject suffering from an inflammatory disease or autimmune disease selected from the group consisting of myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis and inflammatory bowel disease arthritis. The term "anti-TNF therapy" is not to be limited to a

30 known anti-TNF therapy, such as, for example, Etanercept, Remicade or Humira notwithstanding that those anti-TNF therapies are clearly encompassed.

The term "anti-TNF therapy" further includes any recommended dose and route of administration of a known anti-TNF agent, including but not limited to Etanercept,

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Remicade or Humira.

The term "anti-TNF therapy" further encompasses any adjunct therapy in which an effective amount of a known anti-TNF compound (e.g., Etanercept, Remicade or Humira) 5 is administered in combination with a second agent, such as, for example, an immunosuppressant compound e.g., methotrexate, azathioprine or a corticosteroid).

BRIEF DESCRIPTION OF THE DRAWINGS

10   Figure 1a is a graphical representation showing mouse serum BAFF levels by ELISA (n ≥ 4 animals per group, 5 mo of age), in wild type (squares), TNF$^{-/-}$ (diamonds), BAFF-Tg (circles), and TNF$^{-/-}$ BAFF-Tg mice (triangles) (mean indicated by horizontal bar).

Figure 1b is a graphical representation showing splenic weights (mg) of wild type mice (▨ 15   ), TNF$^{-/-}$ mice (▢), BAFF-Tg mice (▩), and TNF$^{-/-}$ BAFF-Tg mice (▢) at 2-4 months, 5-7 months and 11-13 months of age. Data are shown as the mean ± SD for at least eight animals per group.

Figure 1b is a graphical representation showing numbers of CD4 (left panel) and CD8 20   (right panel) T cells in 12-month old wild type mice (▩), TNF$^{-/-}$ mice (▢), BAFF-Tg mice (▨), and TNF$^{-/-}$ BAFF-Tg mice (▢). Data are shown as the mean ± SD for at least eight animals per group.

Figure 1d shows graphical representations of flow cytometry data on splenocytes isolated 25   from 6-month old wild type (WT) mice, TNF$^{-/-}$ mice, BAFF-Tg mice, and TNF$^{-/-}$ BAFF-Tg mice as indicated on the x-axis. Splenocytes from littermates were stained in parallel with a mixture of anti-IgM/anti-CD23/anti-CD21/anti-IgD or anti-CD5/anti-B220/anti-IgM/anti-IgD or anti-B220/anti-Ly-6D antibodies and analyzed by flow cytometry. Various B cell subsets were gated according to their respective phenotype: Top left panel: B220 30   positive B cells (B200^{high}); Row 2 left column panel: B1-a B cells (B220^{Int}, IgM^{high}, CD5^{+}); Row 3 left column panel: B-1b B cells (B220^{Int}, IgM^{high}, CD5^{-}); Row 4 left column: plasmablasts (B220^{low}, Ly-6D^{high}); Top right panel: T1 B cells (CD23^{-}, IgM^{high}, IgD^{-}, CD21^{-}); Row 2 right column panel: T2 B cells (CD23^{+}, IgM^{high}, IgD^{high}, CD21^{high}); Row 3 right column panel: mature B cells (CD23^{+}, IgM^{dull}, IgD^{+}, CD21$^{Intermediate(Int)}$); and Row 4 right 35   column panel: MZ B cells (CD23^{-}, IgM^{high}, IgD^{-}, CD21^{high});   Percentages for all B cell

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subsets were calculated by the flow cytometer, and mean absolute numbers per spleen ± SD for five to nine animals are shown. Statistical analysis was done using ANOVA, and significance with relation to WT is indicated above each histogram, as follows: $p < 0.001$ (...), $p < 0.005$ (..), $p < 0.05$ (.).

Figure 2a provides graphical representations showing that T-dependent immune responses are impaired in TNF$^{-/-}$ BAFF-Tg mice, while TI-2 responses are increased. Wild type (WT; □), TNF$^{-/-}$ (◇◇), BAFF-Tg (○), and TNF$^{-/-}$ BAFF-Tg (△) littermates were immunized with SRBC, and SRBC-specific IgM (upper panel) and IgG (lower panel) antibody responses were determined 7 and 14 days post-immunization using SRBC hemagglutination assays. Six animals were analyzed per group. Data include the mean (horizontal bars) and show that anti-SRBC IgM concentrations after 14 days (upper right panel) and IgG (lower panels) responses were significantly reduced in TNF$^{-/-}$ and TNF$^{-/-}$ BAFF-Tg mice compared with WT or BAFF-Tg littermates ($p < 0.05$ by ANOVA).

Figure 2b is a graphical representation showing NP-specific antibody responses in NP-OVA-immunized (WT; □), TNF$^{-/-}$ (◇◇), BAFF-Tg (○), and TNF$^{-/-}$ BAFF-Tg (△) littermates 7 days after a secondary immunization with NP-OVA. At least seven animals per group were analyzed. The mean antibody production for each panel is indicated by the horizontal bars. No significant difference was observed between TNF$^{-/-}$ mice and TNF$^{-/-}$ mice or BAFF-Tg mice, or between WT mice and BAFF-Tg mice ($p > 0.05$).

Figure 2c shows graphical representations of NP-specific antibody responses in 6-month old wild type (WT; ■), TNF$^{-/-}$ (□), BAFF-Tg (▣), and TNF$^{-/-}$ BAFF-Tg (▢) mice 7 days after immunization with TI-2 NP-Ficoll. Data indicate total Ig (top panel), IgM (second panel from top), IgG3 (third panel from top) and IgA (bottom panel) responses. Six animals per group were analysed. Statistical analysis was done using ANOVA, and significance with relation to WT is indicated at the top of each histogram, as follows: $p < 0.001$ (...), $p < 0.005$ (..), and $p < 0.05$ (.).

Figure 3a provides graphical representations showing levels of autoantibodies against RF (left panel), anti-dsDNA (middle panel) and anti-ssDNA (right panel) in 12-month old mice, as determined by specific ELISA. Animals were either wild-type (WT; squares), TNF$^{-/-}$ (diamonds), BAFF-Tg (circles), or TNF$^{-/-}$ BAFF-Tg (triangles). The mean autoantibody production for each panel is shown with horizontal bars. Data indicate that TNF$^{-/-}$ BAFF-Tg mice produce high levels of RF and anti-DNA autoantibodies.

Figure 3b is a copy of photographic representations of representative H&E-stained 5-μm kidney tissue sections from 12-month old wild type mice (wt; upper left panel), TNF$^{-/-}$ mice (upper right panel), BAFF-Tg mice (lower left panel) and TNF$^{-/-}$ BAFF-Tg mice (lower right

5   panel). More than 30 mice were dissected from each group. Arrows indicate glomeruli. Data show that TNF$^{-/-}$ BAFF-Tg mice develop nephritis and Sjögren's-like pathologies.

Figure 3c provides copies of photographic representations of representative H&E stained submaxillary gland sections from 12-month old wild type mice (WT; left panel), TNF$^{-/-}$ mice

10   (second panel from left), BAFF-Tg mice (third panel from left) and TNF$^{-/-}$ BAFF-Tg mice (right panel). More than 30 mice were dissected from each group. Arrows show the presence of normal ducts and acinar cells. Data show that TNF$^{-/-}$ BAFF-Tg mice develop nephritis and Sjögren's-like pathologies.

15   Figure 4a provides copies of photographic representations of cervical lymphoid masses (CLN as indicated by arrows) associated with submaxillary glands in two TNF$^{-/-}$ BAFF-Tg mice (right panels) compared with BAFF-Tg mice salivary glands (left panel).

Figure 4b provides copies of photographic representations of cervical lymphoid tumor in a

20   TNF$^{-/-}$ BAFF-Tg mouse (right) compared with a typically enlarged BAFF-Tg cervical lymph node (LN) (left).

Figure 4c is a copy of a photographic representation of a lymphoma of the small intestine in a TNF$^{-/-}$ BAFF-Tg mouse (above) compared with unaffected small intestine (below).

25   The dotted line indicates the orientation of a cross section for histology.

Figure 4d provides copies of photographic representations of H&E stained small intestine (left panel) for a wild type animal (WT small intestine; left panels) and a TNF-deficient BAFF-Tg mouse having a small intestine lesion (TNF$^{-/-}$.BAFF-Tg small intestine; right

30   panels). Respective boxed regions in the upper panels are shown at higher magnification in the corresponding lower panels. Arrow indicates residual mucosa and lymphoepithelial damage in the TNF$^{-/-}$ BAFF-Tg mouse small intestine lesion (lower right panel).

Figure 4e provides copies of photographic representations of H&E staining of mesenteric

35   lymphoid masses from wild type (WT MLN; left panels) and a TNF$^{-/-}$ BAFF-Tg mouse (right panels) at 10X magnification (upper panels) and 40X magnification (lower panels).

The arrow in the upper left panel indicates a follicle within the WT MLN. No lymphoid microarchitecture was recognizable in the MLN mass found in the TNF$^{-/-}$ BAFF-Tg mouse. Cells in the mesenteric lymphoid mass found in a TNF$^{-/-}$ BAFF-Tg mouse display neoplastic features such as irregular nucleus, clumped chromatin, and increased

5   cytoplasmic volume.

Figure 5 is a graphical representation showing that cervical lymph node (CLN) masses and mesenteric lymph node (MLN) masses contained very large numbers of lymphocytes.   Cell number per LN was determined by hemocytometer counts on

10 mechanically-disrupted nodes. Control LNs were from similarly aged mice.

Figure 6 is a graphical representation showing lymphocyte proportions (B cells, CD4+ T cells and CD8+ T cells) in mesenteric lymph node (MLN) masses.

15 Figure 7 is a graphical representation showing lymphocyte proportions (B cells, CD4+ T cells and CD8+ T cells) in salivary gland lymph nodes.

Figure 8 provides graphical representations showing reduced TACI up-regulation in B cells from TNF$^{-/-}$ BAFF-Tg mice. Splenocytes from wild type mice (wt; top panel), TNF$^{-/-}$

20 mice (second panel from top), BAFF-Tg mice (third panel from top), and TNF$^{-/-}$ BAFF-Tg mice (bottom panel) were activated for up to 48 h with goat anti-μ antibodies. Mean fluorescence intensity (MFI) of TACI expression over time following activation is shown on gated B220^{+} B cells from wt, TNF$^{-/-}$, BAFF-Tg, and TNF$^{-/-}$ BAFF-Tg mice, as indicated, as determined using flow cytometry to detect an anti-mouse TACI antibody. The data

25 indicate representative results of three separate experiments.

DETAILED DESCRIPTION OF THE INVENTION
One aspect of the present invention provides nucleic acid-based methods and protein-based methods for diagnosing cancer in a human or other mammal. More particularly the

30 present invention provides a method of diagnosing a predisposition for a neoplastic disease in a human or animal subject said method comprising determining the level of BAFF protein in a biological sample derived from the subject wherein an elevated level of BAFF protein in the sample is indicative of a predisposition towards the neoplastic disease.

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As used herein, the tem "BAFF protein" shall be taken to mean any peptide, polypeptide, or protein having at least about 65% amino acid sequence identity to an amino acid sequence a human or mouse BAFF polypeptide set forth in SEQ ID NO: 2 or 4. The term "BAFF protein" shall also be taken to include a peptide, polypeptide or protein having the

5   known biological activity of an exemplified BAFF protein, or the known binding specificity of an exemplified BAFF protein. For the purposes of nomenclature, the amino acid sequences of the human and mouse BAFF polypeptides are exemplified herein, as SEQ ID Nos: 2 and 4, respectively. Preferably, the percentage identity to SEQ ID NO: 2 or SEQ ID NO: 4 is at least about 80%, more preferably at least about 90%, even more

10 preferably at least about 95% and still more preferably at least about 99%. In a particularly preferred embodiment, the BAFF protein is a human BAFF protein having at least about 95% identity to SEQ ID NO: 2.

In determining whether or not two amino acid sequences fall within these defined

15 percentage identity limits, those skilled in the art will be aware that it is necessary to conduct a side-by-side comparison of amino acid sequences. In such comparisons or alignments, differences will arise in the positioning of non-identical amino acid residues depending upon the algorithm used to perform the alignment. In the present context, references to percentage identities and similarities between two or more amino acid

20 sequences shall be taken to refer to the number of identical and similar residues respectively, between said sequences as determined using any standard algorithm known to those skilled in the art. In particular, amino acid identities and similarities are calculated using the GAP program of the Computer Genetics Group, Inc., University Research Park, Madison, Wisconsin, United States of America (Devereaux et al, Nucl.

25 Acids Res. 12, 387-395,1984), which utilizes the algorithm of Needleman and Wunsch J. Mol. Biol. 48, 443-453, 1970, or alternatively, the CLUSTAL W algorithm of Thompson et al., Nucl. Acids Res. 22, 4673-4680, 1994, for multiple alignments, to maximize the number of identical/similar amino acids and to minimize the number and/or length of sequence gaps in the alignment.

30

For the purposes of nomenclature, the nucleotide sequences set forth in SEQ ID Nos: 1 and 3 relate to nucleic acids that encode the human and murine BAFF proteins, respectively, the amino acid sequences of which are set for the as SEQ ID Nos: 2 and 4.

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As used herein, the term "diagnosis", and variants thereof, such as, but not limited to "diagnose", "diagnosed" or "diagnosing" shall not be limited to a primary diagnosis of a clinical state, however should be taken to include any primary diagnosis of a predisposition for disease or a diagnosis of an actual disease state or a prognosis of a clinical state. For example, the "diagnostic assay" formats described herein are equally relevant to assessing the remission of a patient, or monitoring disease recurrence, or tumor recurrence, such as following surgery or chemotherapy, or determining the appearance of metastases of a primary tumor. All such uses of the assays described herein are encompassed by the present invention.

Those skilled in the art will be aware that as a cancer progresses, metastases occur in organs and tissues outside the site of the primary tumor. For example, in the case of ovarian cancer, metastases commonly appear in a tissue selected from the group consisting of omentum, abdominal fluid, lymph nodes, lung, liver, brain, and bone. The present invention clearly contemplates the use of BAFF over expression, particularly in blood or serum or a derivative thereof, for diagnosing or detecting any stage of progression of a circulating or solid cancer, including early stages of the disease and metastases thereof.

Moreover, as the present invention is particularly useful for diagnosing a predisposition for cancer in a subject, the diagnostic method described herein is not to be limited by the stage of a cancer in the subject from which the biological sample is derived (ie. whether or not the patient is in remission or undergoing disease recurrence or whether or not the cancer is a primary tumor or the consequence of metastases).

Preferably, the subject is an individual receiving anti-TNF therapy or being considered for anti-TNF therapy. The rationale for anti TNFα therapy has been documented and published in many reviews (eg., Feldmann and Maini, *Ann. Rev. Immunol.*, 2001; Bondeson and Maini, *Int. J. Clin. Pract.*, 2001). Anti-TNF compounds include infliximab (in adults) and etanercept (in adults and children). Infliximab is a mouse-derived, humanised IgG monoclonal antibody, while etanercept is a soluble receptor. Anti-TNF therapy is indicated for several inflammatory disorders, such as, for example, rheumatoid arthritis, as well as other diseases such as, for example, Parkinsons Disease, Crohn's Disease, and psoriasis. Clinical trials of anti TNFα antibody therapy in rheumatoid

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arthritis showed clinical benefit in 70% of patients treated with 3 or 10 mg/kg, which lasted for more than 12 weeks after cessation of therapy. Low doses of antibody are particularly used in combination with methotrexate, as a consequence of synergism between the antibody and a low dose of methotrexate.

Alternatively, or additionally, the subject may be an aged subject. By "aged" is meant a subject of an age at which the onset of rheumatoid conditions, such as, for example, rheumatoid arthritis, are common. Alternatively, or additionally, the subject may be a subject suffering from a rheumatoid condition or other inflammatory condition, such as, for example, rheumatoid arthritis.

The neoplastic diseases contemplated herein to be amenable to diagnosis are solid tumors or circulating cancers, such as, for example, leukemia, lymphoma, myeloma, head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer (eg. gastric, colon, or pancreatic cancer), renal cell cancer, bladder cancer, a gynecological carcinoma (eg. ovarian cancer), prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, epithelial vulval carcinoma or medulloblastoma. The diagnosis of a circulating cancer or a predisposition towards a circulating cancer, such as, for example, a leukemia, lymphoma, or myeloma, is particularly preferred.

Naturally, the biological sample on which the diagnosis is based will depend to some extent upon the nature of the neoplasm being diagnosed. In the case of a circulating cancer, it is especially preferred to make a diagnosis on the basis of BAFF levels mRNA in blood or serum or a fraction thereof (eg., peripheral blood mononuclear cells (PBMC), or buffy coat preparation), or the level of BAFF-encoding mRNA in blood or serum or a fraction thereof (eg., peripheral blood mononuclear cells (PBMC), or buffy coat preparation). In the case of a solid tumor, a diagnosis is generally made by determining BAFF protein level or BAFF-encoding mRNA in a tissue selected from the group consisting of: abdominal fluid, lymph, lung, prostate, omentum, ovary, liver, kidney, spleen, lung, placenta, and brain.

The level of BAFF expression is determined at either the RNA level or the protein level and all such methods are encompassed by the present invention. The present invention

provides both nucleic acid-based assays and immunoassays for detecting a predisposition toward cancer and/or for determining the progress of the disease, particularly in patients receiving anti-TNF therapy or candidates for anti-TNF therapy.

5   Preferably, the test sample used in performing this embodiment of the invention is blood or whole serum or a fraction thereof comprising T cells, such as, for example, buffy coat. Other tissues, such as those described *supra* are not excluded.

Preferably, the level of BAFF polypeptide in the test sample is determined by a process
10 comprising:
    (i)   contacting said sample with an antibody that binds to a BAFF polypeptide under conditions sufficient for binding to occur; and
    (ii)   determining the binding.

15 Preferred protein-based detection systems contemplated herein include any known assay for detecting a protein in a biological sample isolated from a human or mammalian subject, such as, for example, using one or more antibodies against the protein or an epitope thereof. Alternatively, a non-antibody ligand of the protein may be used, such as, for example, a small molecule (e.g. a chemical compound, agonist, antagonist, allosteric
20 modulator, competitive inhibitor, or non-competitive inhibitor that may or may not modulate activity of the protein).

The protein-based assays described herein are generally immunoassays that utilize antibodies, including monoclonal and polyclonal antibodies, or a Fab fragment, F(ab')$_2$
25 fragment, or scFv fragment, that bind to a unique region comprising at least about 5-10 contiguous amino acid residues of a BAFF polypeptide (eg., the less highly conserved N-terminal hydrophilic region). Regions of the murine BAFF polypeptide that are highly conserved with the human sequence (eg. any peptide comprising at least about 5-10 contiguous amino acid residues of SEQ ID No: 2 or SEQ ID NO: 4 are particularly useful
30 for preparing antibodies against human BAFF.

Antibody-based assay systems are particularly preferred. In accordance with these embodiments, the antibody or small molecule may be used in any standard solid phase or solution phase assay format amenable to the detection of proteins or portions thereof.

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Antibodies that specifically bind to the BAFF protein are used for the diagnosis of conditions or diseases characterized by the presence of said protein, or in prognostic assays to monitor disease progression in the presence of absence of treatment.

5

In one embodiment, preferred antibodies for diagnostic applications will bind to at least about 5 contiguous amino acids in the region from position 1 to about position 200 of a BAFF protein, more preferably at least about 5 contiguous amino acids in the region from position 1 to about position 180 of a BAFF protein and still more preferably at least about

10   5 contiguous amino acids in the region from position 1 to about position 170 of a BAFF protein, and even still more preferably at least about 5 contiguous amino acids in the region from position 1 to about position 150 of a BAFF protein. This embodiment takes advantage of the greater divergence at the N-terminus of a BAFF protein from other members of the TNF family of proteins, compared to the C-terminal portion of the protein.

15

In another embodiment, preferred antibodies for use in diagnostics are those which react with an epitope in an extracellular region of the BAFF polypeptide. Such antibodies may be generated by using the complete human BAFF polypeptide (SEQ ID NO: 2) as an immunogen. Alternatively, a peptide fragment derived from a predicted extracellular

20   domain thereof, can be used. In this regard, the region of the of the polypeptide that is N-terminal to the first transmembrane domain, or a peptide fragment thereof, may be selected and screened for its ability to elicit the production of extracellular-specific anti-BAFF antibodies using standard immunoassays, such as, for example, ELISA.

25   Such antibodies are available commercially (eg., Research Diagnostics, Inc., Pleasant Hill Road Flanders, NJ 07836, USA). An antibody prepared against a peptide corresponding to amino acids 254 to 269 of human BAFF, that cross-reacts with murine and human BAFF proteins, is also available from Abcam Ltd,, Hartford, CT 06150-2631, USA. A monoclonal antibody preparation against amino acid residues 83-285 of human BAFF

30   protein is also commercially available from Oncogene Research Products, San Diego, CA92121, USA.

Alternatively, antibodies are prepared against a BAFF protein or an epitope thereof. Conventional methods can be used to prepare the antibodies. For example, by using an

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isolated BAFF polypeptide or immunologically active derivative thereof, polyclonal antisera or monoclonal antibodies can be made using standard methods. For example, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with the polypeptide or peptide to elicit an antibody response in the mammal. Techniques for conferring

5   immunogenicity on a polypeptide include conjugation to carriers, or other techniques well known in the art. For example, the polypeptide can be administered in the presence of adjuvant or can be coupled to a carrier molecule known in the art, that enhances the immunogenicity of the polypeptide. The progress of immunization can be monitored by detection of antibody titres in plasma or serum. Standard ELISA or other immunoassay

10 can be used to assess the titer of antibodies produced. Following immunization, antisera are obtained and, for example, IgG molecules corresponding to the polyclonal antibodies can be isolated from the antisera.

To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be

15 harvested from an animal immunized with the polypeptide or peptide, and fused with myeloma cells by standard somatic cell fusion procedures, thus immortalizing those cells and yielding hybridoma cells. Such techniques are well known in the art, for example, the hybridoma technique originally developed by Kohler and Milstein *Nature* 256: 495-499, 1975, as well as other techniques such as the human B-cell hybridoma technique (Kozbor

20 *et al.*, *Immunol. Today 4:* 72, 1983), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, *In:* Monoclonal antibodies in cancer therapy, Alan R. Bliss Inc., pp 77-96, 1985), and screening of combinatorial antibody libraries (Huse *et al.*, *Science 246*, 1275-1281, 1989). Hybridoma cells are isolated and screened immunochemically for production of antibodies that are specifically reactive with the

25 polypeptide and monoclonal antibodies isolated therefrom.

As with all immunogenic compositions for eliciting antibodies, the immunogenically effective amounts of the peptides of the invention must be determined empirically. Factors to be considered include the immunogenicity of the native polypeptide, whether or

30 not the polypeptide will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier, the route of administration for the composition, ie., intravenous, intramuscular, subcutaneous, *etc.*, and the number of immunizing doses to be administered. Such factors are known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue experimentation.

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The term "antibody" as used herein, is intended to include fragments thereof which are also specifically reactive with a polypeptide that mimics or cross-reacts with a B-cell epitope of a BAFF polypeptide. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')$_2$ fragments can be generated by treating antibody with pepsin. The resulting F(ab')$_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

Diagnostic assays for include methods which utilize the antibody and a label to detect the BAFF protein in human body fluids or extracts of cells or tissues. The antibodies are used with or without modification, and may be labeled, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules which are known in the art may be used, several of which are described above.

A variety of immunoassay protocols, including ELISA, RIA, and FACS, for measuring the amount of BAFF protein in a biological sample are known in the art or described herein. Such methods provide a basis for diagnosing levels of the protein associated with disease. For example, a high serum BAFF level is associated with cancers, particularly those induced during anti-TNF therapy or as a consequence of aging induced by over expression of BAFF (eg. a leukemia, head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer,. gastric cancer, colon cancer, pancreatic cancer, renal cell cancer, bladder cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, medulloblastoma and ER-negative breast cancer). The presence of high serum BAFF levels is indicative of a predisposition to disease and/or a poor prognosis of survival from disease.

For the purposes of determining what constitutes a high or elevated level of BAFF protein in a sample, the skilled artisan will be aware that this is generally achieved by comparison of the test sample to a sample from a healthy or normal subject, such as, for example, a subject having a low risk of developing the disease and who is asymptomatic. Normal or standard values of serum BAFF for a healthy individual are established by combining body fluids or cell extracts taken from one or more normal or healthy subjects, preferably

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human subjects.

In the present context, the term "healthy individual" shall be taken to mean an individual who is known not to suffer from cancer and/or does not have elevated BAFF expression,
5   such knowledge being derived from clinical data on the individual, including, but not limited to, a different cancer assay to that described herein, or a determination of serum BAFF levels for the subject. As the present invention is particularly useful for the early detection of cancer or a predisposition for cancer, it is preferred that the healthy individual is asymptomatic with respect to the early symptoms associated with a particular cancer.
10

For diagnosis of a subject that has undergone or commenced anti-TNF therapy, a "healthy individual" that serves a a suitable control for such a diagnosis includes an individual that has not commenced and preferably has not previously undergone anti-TNF therapy.
15

The term "normal individual" shall be taken to mean an individual having a normal level of BAFF expression similar to that seen in a healthy individual. As will be known to those skilled in the art, data obtained from a sufficiently large sample of the population will normalize, allowing the generation of a data set for determining the average level of a
20   particular parameter. Accordingly, the level of expression of BAFF can be determined for any population of individuals, and for any sample derived from said individual, for subsequent comparison to BAFF levels determined for a sample being assayed. Where such normalized data sets are relied upon, internal controls are preferably included in each assay conducted to control for variation.
25

The amount of standard BAFF protein is quantified by various methods, preferably by photometric means, or using antibodies in a quantitative immunoassay (e.g. ELISA), wherein the amount of protein is determined by comparison against known amounts of a standard peptide, such as, for example, a peptide comprising a portion of the full length
30   BAFF protein. Preferably, the N-terminal portion of the BAFF protein is used, to achieve enhanced specificity.

Quantities of the protein expressed in subject samples from biopsied tissues or body fluids, particularly serum or blood or a fraction thereof, are compared with the standard or

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normal values. Deviation between standard and subject values establishes the parameters for diagnosing disease or establishing a prognosis. More particularly, a level of a BAFF protein in excess of the standard level of that protein detected in a healthy subject, is diagnostic of a predisposition for disease, and/or indicates a poor prognosis

5 for survival.

The use of detections systems such as, for example, optical or fluorescent detection, mass spectrometry, MALDI-TOF, biosensor technology, evanescent fiber optics, or fluorescence resonance energy transfer, is clearly encompassed by the present invention.

10

In biosensor diagnostic devices, the assay substrate and detector surface are integrated into a single device. One general type of biosensor employs an electrode surface in combination with current or impedance measuring elements for detecting a change in current or impedance in response to the presence of a protein-protein binding event (e.g.

15 U.S. Patent No. 5,567,301). Gravimetric biosensors employ a piezoelectric crystal to generate a surface acoustic wave whose frequency, wavelength and/or resonance state are sensitive to surface mass on the crystal surface. The shift in acoustic wave properties is therefore indicative of a change in surface mass, such as, for example, as a consequence of protein-protein binding (e.g. U.S. Patent Nos. 5,478,756 and 4,789,804.

20 Biosensors based on surface plasmon resonance (SPR) effects have also been proposed, for example, in U.S. Patent Nos. 5,485,277 and 5,492,840, which exploit the shift in SPR surface reflection angle that occurs when protein binds to the SPR interface. Finally, a variety of biosensors that utilize changes in optical properties at a biosensor surface are known, (e.g., U.S. Patent No. 5,268,305).

25

Biosensors have a number of potential advantages over binding assay systems having separate reaction substrates and reader devices. One important advantage is the ability to manufacture small-scale, but highly reproducible, biosensor units using microchip manufacturing methods, such as, for example, described in U.S. Patent Nos. 5,200,051

30 and 5,212,050. Another advantage is the potentially large number of different analyte detection regions that can be integrated into a single biosensor unit, allowing sensitive detection of several analytes with a very small amount of body-fluid sample. Accordingly, the simultaneous detection of the individual binding partners that form the protein, or the simultaneous detection of one or more proteins of the present invention, is possible using

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a biosensor.

Evanescent biosensors are particularly preferred because they do not require separation of the protein from unbound material, and their use can be coupled to standard immunoassay formats, as originally described by Hirshfield in U.S. Patent No. 4,447,546. In general, evanescent biosensors rely upon light of a particular wavelength interacting with a fluorescent molecule, such as, for example, a fluorescent antibody or small molecule attached near the probe's surface, to emit fluorescence at another wavelength, on binding of the protein of the invention to the antibody or small molecule. The biosensor is protected from sensitivity degradation caused by non-specific binding of proteins to the sensor surface, by exposing the sensor surface to a solution of non-interfering proteins, so that the non-interfering proteins bind to said sensor surface to prevent the subsequent binding of the interfering proteins. Enhanced protection of surfaces from biological proteins is also possible by completely covering surfaces with protective coatings, such as, for example, amorphous copolymers of tetrafluoroethylene and bis-2,2-trifluoromethyl-4.5-difluoro-1,2-dioxole, dissolved in a solvent containing fluorinated alkanes, and applied by deposition as a thin protective coating (US. Patent No. 5,356,668 by Paton *et al.*).

Assay systems suitable for use in high throughput screening of mass samples, particularly a high throughput spectroscopy resonance method (e.g. MALDI-TOF, electrospray MS or nano-electrospray MS) or a detection system facilitating determination of real time association/dissociation constants of antigen-antibody complexes, are particularly contemplated.

In an alternative embodiment, a biological sample derived from a subject, such as, for example, blood or serum or a B cell-containing fraction thereof, is assayed for the presence of elevated levels of anti-BAFF antibodies. In general, such assays determine the ability of the sample to block the binding of a BAFF protein to a cell that expresses a receptor to which BAFF protein binds. An example of such an assay is provided, for example, by Biogen, inc. (WO 99/12964).

In an alternative embodiment, the present invention provides a nucleic acid-based diagnostic assay for determining a predisposition toward a cancer in a subject, or for

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diagnosing cancer or determining a cancer cell. As used herein, the term "nucleic acid" shall be taken to mean any single-stranded or double-stranded RNA, DNA, cDNA, cRNA, or synthetic oligonucleotide, or alternatively, an analog of RNA, DNA, cDNA, cRNA, or a synthetic oligonucleotide. "Nucleic acid" also includes any genomic gene equivalents of a
5   cDNA molecule.

In a preferred embodiment, the isolated nucleic acid of the invention is from humans (ie. it encodes a human BAFF polypeptide).

10   More particularly, the nucleic acid-based assays described herein rely upon the detection or relative quantification of RNA levels in samples using probes of at least about 20 nucleotides in length that hybridize specifically to RNA encoding the BAFF polypeptide, or alternatively, amplify cDNA from RNA encoding the BAFF polypeptide. Such probes are derived from unique regions of any one or more of the BAFF-encoding genes described
15   herein, such as, for example, any 15-20 contiguous nucleotides within a sequence having at least about 80% identity to SEQ ID NO: 1 or 3 or a sequence having at least about 80% identity to the protein-encoding region of SEQ ID NO: 1 or 3 or a complementary nucleotide sequence thereto or identical sequence in any other BAFF-encoding gene. The use of full-length antisense cDNA or cRNA derived from a BAFF-encoding gene such
20   as, for example a gene comprising the nucleotide sequence of SEQ ID No: 1 is also encompassed by the present invention. However, as the 3'-end of the protein-encoding region of a BAFF gene is highly conserved relative to the 5'-end of the protein coding region, it is particularly preferred to use probes that specifically hybridize to a non-coding region of BAFF mRNA (ie., the 5'-UTR or 3'UTR), or alternatively or in addition, that
25   hybridize specifically to the 3'-end of the protein-encoding region of a BAFF gene. Preferably, a probe that hybridizes to the protein-encoding region of BAFF mRNA will hybridize to a region that encodes at least about 5 contiguous amino acids in the region from position 1 to about position 200 of a BAFF protein, more preferably at least about 5 contiguous amino acids in the region from position 1 to about position 180 of a BAFF
30   protein and still more preferably at least about 5 contiguous amino acids in the region from position 1 to about position 170 of a BAFF protein, and even still more preferably at least about 5 contiguous amino acids in the region from position 1 to about position 150 of a BAFF protein.

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Preferred nucleic acid probes for detecting RNA encoding a BAFF polypeptide in a sample may comprise double-stranded or single-stranded nucleic acid. Single-stranded probes are preferred because they do not require melting prior to use in hybridizations. On the other hand, longer probes are also preferred because they can be used at higher
5   hybridization stringency than shorter probes and may produce lower background hybridization than shorter probes.

So far as shorter probes are concerned, single-stranded, chemically-synthesized oligonucleotide probes are particularly preferred by the present invention. To reduce the
10 noise associated with the use of such probes during hybridization, the nucleotide sequence of the probe is carefully selected to maximize the Tm at which hybridizations can be performed, reduce non-specific hybridization, and to reduce self-hybridization. Such considerations may be particularly important for applications involving high throughput screening using microarray technology. In general, this means that the
15 nucleotide sequence of an oligonucleotide probe is selected such that it is unique to BAFF RNA or BAFF protein-encoding sequence, has a low propensity to form secondary structure, low self-complementary, and is not highly A/T-rich.

The only requirement for the probes is that they cross-hybridize to nucleic acid encoding
20 BAFF or the complementary nucleotide sequence thereto and are sufficiently unique in sequence to generate high signal:noise ratios under specified hybridization conditions. As will be known to those skilled in the art, long nucleic acid probes are preferred because they tend to generate higher signal:noise ratios than shorter probes and/or the duplexes formed between longer molecules have higher melting temperatures (ie. Tm values) than
25 duplexes involving short probes. Accordingly, full-length DNA or RNA probes are contemplated by the present invention, as are specific probes comprising the sequence of the 3'-untranslated region or complementary thereto.

In a particularly preferred embodiment, the nucleotide sequence of an oligonucleotide
30 probe has no detectable nucleotide sequence identity to a nucleotide sequence in a BLAST search (Altschul et al., J. Mol. Biol. 215, 403-410, 1990) or other database search, other than a sequence selected from the group consisting of: (a) a sequence encoding a human BAFF polypeptide; (b) the 5'-untranslated region of a sequence encoding a human

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BAFF polypeptide; (c) a 3'-untranslated region of a sequence encoding a human BAFF polypeptide; and (d) an exon region of a sequence encoding a human BAFF polypeptide.

Even more preferably, the nucleotide sequence of an oligonucleotide probe has the
5   following properties:
  (i)   it comprises less than ten(10) A residues;
  (ii)   it comprises less than ten(10) T residues;
  (iii)   it comprises less than nine(9) C residues;
  (iv)   it comprises less than nine(9) G residues;
10 (v)   it comprises less than seven(7) A residues in any window consisting of 8
          nucleotides;
  (vi)   it comprises less than seven(7) T residues in any window consisting of 8
          nucleotides;
  (vii)   it comprises less than eight(8) C residues in any window consisting of 8
15   nucleotides;
  (viii) it comprises less than eight(8) G residues in any window consisting of 8
          nucleotides;
  (ix)   it comprises less than six(6) consecutive A residues;
  (x)   it comprises less than six(6) consecutive T residues;
20 (xi)   it comprises less than five(5) consecutive C residues; and
  (xii)   it comprises less than five(5) consecutive G residues.

Additionally, the self-complementarity of a nucleotide sequence can be determined by aligning the sequence with its reverse complement, wherein detectable regions of identity
25 are indicative of potential self-complementarity. As will be known to those skilled in the art, such sequences may not necessarily form secondary structures during hybridization reaction, and, as a consequence, successfully identify a target nucleotide sequence. It is also known to those skilled in the art that, even where a sequence does form secondary structures during hybridization reactions, reaction conditions can be modified to reduce
30 the adverse consequences of such structure formation. Accordingly, a potential for self-complementarity should not necessarily exclude a particular candidate oligonucleotide from selection. In cases where it is difficult to determine nucleotide sequences having no potential self-complementarity, the uniqueness of the sequence should outweigh a consideration of its potential for secondary structure formation.

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Recommended pre-requisites for selecting oligonucleotide probes, particularly with respect to probes suitable for microarray technology, are described in detail by Lockhart *et al.*, "Expression monitoring by hybridization to high-density oligonucleotide arrays", 5   *Nature Biotech.14*, 1675-1680, 1996.

The nucleic acid probe may comprise a nucleotide sequence that is within the coding strand of the BAFF-encoding gene (ie. it is comprised within the nucleotide sequence of RNA encoding BAFF). Such "sense" probes are useful for detecting RNA encoding BAFF 10   by amplification procedures, such as, for example, polymerase chain reaction (PCR), and more preferably, quantitative PCR or reverse transcription polymerase chain reaction (RT-PCR). Alternatively, "sense" probes may be expressed to produce BAFF polypeptides or immunologically active derivatives thereof that are useful for detecting the expressed BAFF protein in samples.

15

Alternatively, a probe is designed from within the antisense strand of said gene (ie. it is complementary to RNA encoding BAFF). Such "antisense" probes are useful for directly hybridizing to RNA encoding BAFF, or alternatively, for detecting RNA encoding BAFF by amplification, as described *supra* (eg. quantitative PCR or RT-PCR).

20

In one particularly preferred embodiment, the present invention provides a method for diagnosing a predisposition toward cancer in a subject, said method comprising:

(i)   determining the level of BAFF mRNA expressed in a test sample from said subject; and

25   (ii)   comparing the level of BAFF mRNA determined at (i) to the level of BAFF mRNA expressed in a comparable sample from a healthy or normal individual, wherein a level of BAFF mRNA at (i) that is enhanced in the test sample relative to the comparable sample from the normal or healthy individual is indicative of the presence of a cancer cell in said subject.

30

By "BAFF mRNA" is meant mRNA encoding a BAFF polypeptide that has at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3or a complementary sequence thereto, and, more particularly, mRNA comprising a nucleotide sequence that has at least about 80% identity, more preferably at least about 90% identity, and still more preferably at

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least about 95% or 99% identity to the nucleotide sequence set forth in SEQ ID NO: 1 or
SEQ ID NO: 3 or a complementary sequence thereto.

5   In a preferred embodiment, the level of BAFF mRNA in the test sample is determined by
hybridizing a BAFF probe to BAFF-encoding RNA in the test sample under at least low
stringency hybridization conditions and detecting the hybridization using a detection
means.

Similarly, the level of BAFF mRNA in the comparable sample from the healthy or normal
10   individual is preferably determined by hybridizing a BAFF probe to BAFF-encoding RNA
in said comparable sample under at least low stringency hybridization conditions and
detecting the hybridization using a detection means.

Preferably, the sample comprises blood, serum, PBMC, buffy coat, ovarian tissue,
15   prostate tissue, kidney tissue, uterine tissue, placenta, a cervical specimen, omentum,
rectal tissue, brain tissue, bone tissue, lung tissue, lymphatic tissue, urine, semen,
abdominal fluid, or a cell preparation or nucleic acid preparation derived therefrom. The
sample can be prepared on a solid matrix for histological analyses, or alternatively, in a
suitable solution such as, for example, an extraction buffer or suspension buffer, and the
20   present invention clearly extends to the testing of biological solutions thus prepared.

The detection means according to this aspect of the invention may be any nucleic acid-
based detection means such as, for example, nucleic acid hybridization or amplification
reaction (eg. PCR), a nucleic acid sequence-based amplification (NASBA) system,
25   inverse polymerase chain reaction (iPCR), in situ polymerase chain reaction, or reverse
transcription polymerase chain reaction (RT-PCR) such as, for example, performed on
laser capture microdissected samples, in situ hybridization, northern blotting techniques,
and microarray technology, such as, for example, using tissue microarrays probed with
nucleic acid probes, or nucleic acid microarrays (ie. RNA microarrays or amplified DNA
30   microarrays) microarrays probed with nucleic acid probes. All such assay formats are
encompassed by the present invention.

For high throughput screening of large numbers of samples, such as, for example, public
health screening of subjects, particularly human subjects having a higher risk of

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developing cancer, microarray technology is a preferred assay format. In further embodiments, an oligonucleotide derived from a nucleotide sequence encoding any one or more of the binding partners is used as a target in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously, to

5   produce a transcript image, or to identify a genetic variant, mutant, or polymorphism for the protein of the invention. In one embodiment, the microarray is prepared and used according to the methods described by Chee *et al.*, WO95/11995, Lockhart *et al.*, *Nat. Biotech. 14*, 1675-1680, 1996, or Schena *et al.*, *Proc. Natl. Acad. Sci. USA 93*, 10614-10619, 1996. A microarray is preferably composed of a large number of unique, single-

10   stranded nucleic acid sequences, such as, for example, synthetic antisense oligonucleotides or fragments of cDNAs, fixed to a solid support. The oligonucleotides are preferably about 6-60 nucleotides in length, more preferably 15-30 nucleotides in length, and most preferably about 20-25 nucleotides in length. Preferred oligonucleotides have a GC content within a range suitable for hybridization, and lack predicted secondary

15   structure that may interfere with hybridization. In certain situations it may be appropriate to use pairs of oligonucleotides containing single nucleotide mismatches, to control for non-specific hybridization/amplification. To conduct sample analysis using a microarray, the RNA or DNA from a biological sample is made into hybridization probes. The mRNA is isolated, and cDNA is produced and used as a template to make antisense RNA

20   (aRNA). The aRNA is amplified in the presence of fluorescent nucleotides, and labeled probes are incubated with the microarray so that the probe sequences hybridize to complementary oligonucleotides of the microarray. Incubation conditions are adjusted so that hybridization occurs with precise complementary matches or with various degrees of less complementarity. After removal of non-hybridized probes, a scanner is used to

25   determine the levels and patterns of fluorescence. The scanned images are examined to determine degree of complementarity and the relative abundance of each oligonucleotide sequence on the microarray. The biological samples may be obtained from any bodily fluids (such as blood, urine, saliva, phlegm, gastric juices, etc.), cultured cells, biopsies, or other tissue preparations. A detection system is used to measure the absence, presence,

30   and amount of hybridization for all of the distinct sequences simultaneously.

For the purposes of defining the level of stringency to be used in these diagnostic assays, a low stringency is defined herein as being a hybridization and/or a wash carried out in 6xSSC buffer, 0.1% (w/v) SDS at 28°C, or equivalent conditions. A moderate stringency

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is defined herein as being a hybridization and/or washing carried out in 2xSSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C, or equivalent conditions. A high stringency is defined herein as being a hybridization and/or wash carried out in 0.1xSSC buffer, 0.1% (w/v) SDS, or lower salt concentration, and at a temperature of at
5   least 65°C, or equivalent conditions. Reference herein to a particular level of stringency encompasses equivalent conditions using wash/hybridization solutions other than SSC known to those skilled in the art.

Generally, the stringency is increased by reducing the concentration of SSC buffer,
10 and/or increasing the concentration of SDS and/or increasing the temperature of the hybridization and/or wash. Those skilled in the art will be aware that the conditions for hybridization and/or wash may vary depending upon the nature of the hybridization matrix used to support the sample RNA, or the type of hybridization probe used.

15 In general, the sample or the probe is immobilized on a solid matrix or surface (e.g., nitrocellulose). For high throughput screening, the sample or probe will generally comprise an array of nucleic acids on glass or other solid matrix, such as, for example, as described in WO 96/17958. Techniques for producing high density arrays are described, for example, by Fodor *et al.*, Science 767-773, 1991, and in U.S. Pat. No. 5,143,854.
20 Typical protocols for other assay formats can be found, for example in Current Protocols In Molecular Biology, Unit 2 (Northern Blotting), Unit 4 (Southern Blotting), and Unit 18 (PCR Analysis), Frederick M. Ausubul *et al.* (ed)., 1995.

The probe can be labelled with a reporter molecule capable of producing an identifiable
25 signal (e.g., a radioisotope such as ^{32}P or ^{35}S, or a fluorescent or biotinylated molecule). According to this embodiment, those skilled in the art will be aware that the detection of said reporter molecule provides for identification of the probe and that, following the hybridization reaction, the detection of the corresponding nucleotide sequences in the sample is facilitated. Additional probes can be used to confirm the assay results obtained
30 using a single probe.

Wherein the detection means is an amplification reaction such as, for example, a polymerase chain reaction or a nucleic acid sequence-based amplification (NASBA) system or a variant thereof, one or more nucleic acid probes molecules of at least about

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20 contiguous nucleotides in length is hybridized to mRNA encoding BAFF, or alternatively, hybridized to cDNA or cRNA produced from said mRNA, and nucleic acid copies of the template are enzymically-amplified.

5   Those skilled in the art will be aware that there must be a sufficiently high percentage of nucleotide sequence identity between the probes and the RNA sequences in the sample template molecule for hybridization to occur. As stated previously, the stringency conditions can be selected to promote hybridization.

10   In one format, PCR provides for the hybridization of non-complementary probes to different strands of a double-stranded nucleic acid template molecule (ie. a DNA/RNA, RNA/RNA or DNA/DNA template), such that the hybridized probes are positioned to facilitate the 5'-to 3' synthesis of nucleic acid in the intervening region, under the control of a thermostable DNA polymerase enzyme. In accordance with this embodiment, one
15   sense probe and one antisense probe as described herein would be used to amplify DNA from the hybrid RNA/DNA template or cDNA.

In the present context, the cDNA would generally be produced by reverse transcription of mRNA present in the sample being tested (ie. RT-PCR). RT-PCR is particularly useful
20   when it is desirable to determine expression of a BAFF-encoding gene. It is also known to those skilled in the art to use mRNA/DNA hybrid molecules as a template for such amplification reactions, and, as a consequence, first strand cDNA synthesis is all that is required to be performed prior to the amplification reaction.

25   Variations of the embodiments described herein are described in detail by McPherson *et al.*, PCR: A Practical Approach. (series eds, D. Rickwood and B.D. Hames), IRL Press Limited, Oxford. pp1-253, 1991.

The amplification reaction detection means described *supra* can be further coupled to a
30   classical hybridization reaction detection means to further enhance sensitivity and specificity of the inventive method, such as by hybridizing the amplified DNA with a probe which is different from any of the probes used in the amplification reaction.

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Similarly, the hybridization reaction detection means described *supra* can be further coupled to a second hybridization step employing a probe which is different from the probe used in the first hybridization reaction.

5   The comparison to be performed in accordance with the present invention may be a visual comparison of the signal generated by the probe, or alternatively, a comparison of data integrated from the signal, such as, for example, data that have been corrected or normalized to allow for variation between samples. Such comparisons can be readily performed by those skilled in the art.

10

It will be apparent from the subject matter exemplified herein that the oncogenic phenotype of a subject is also detected and may, in some conditions be enhanced, when the level of TNF expression in the subject is reduced or suppressed and BAFF expression in the subject is also elevated. Accordingly, in a preferred embodiment of the various

15   formats of the diagnostic assay described herein, the present invention provides a method of diagnosing a predisposition for a neoplastic disease in a human or animal subject said method comprising:

(i)   determining the level of expression of a BAFF protein or nucleic acid encoding a BAFF protein in a biological sample derived from the subject; and

20   (ii)   determining the level of expression of a TNF protein or nucleic acid encoding a TNF protein in a biological sample derived from the subject

wherein an elevated level of the BAFF protein or nucleic acid encoding the BAFF protein and a reduced level of the TNF protein or nucleic acid encoding the TNF protein in the sample is indicative of a predisposition towards the neoplastic disease.

25

Preferred TNF proteins within the present context are TNF family proteins such as, for example, a protein selected from the group consisting of:

(i)   a TNFα protein comprising an amino acid sequence having at least about 65% identity to the TNFα protein of humans (NCBI Accession No. P01375) or mouse

30   (NCBI Accession No. P06804);

(ii)   an LT-α (TNFβ) protein comprising an amino acid sequence having at least about 65% identity to the LT-α (TNFβ) protein of humans (NCBI Accession No. P01374) or mouse (NCBI Accession No. P09225); and

(iii)   an LT-β (TNFγ) protein comprising an amino acid sequence having at least about

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65% identity to the LT-β (TNFγ) protein of humans (NCBI Accession No. Q06643) or mouse (NCBI Accession No. P41155).

More preferably, the TNF protein detected in accordance with the diagnostic process of the present invention is a TNFα protein comprising an amino acid sequence having at least about 65% identity to the TNFα protein of humans (NCBI Accession No. P01375) or mouse (NCBI Accession No. P06804). By "NCBI" is meant the the database of the National Center for Biotechnology Information at the National Library of Medicine at the National Institutes of Health of the Government of the United States of America, Bethesda, MD, 20894. Preferably, the percentage identity to NCBI Accession No. P01375 or NCBI Accession No. P06804 is at least about 70%, more preferably at least about 80% and still more preferably at least about 90% or 95% or 99%.

Both nucleic acid-based assays and protein-based assays, including antibody-based methods, for determining the level of TNF expression in the sample are contemplated herein.

Assays systems for determining the level of TNF in a biological sample, are known and either publicly available or described in the literature. For example, anti-TNFα antibodies and peptides and assays employing anti-TNFα antibodies and peptides for detecting TNF in biological samples are described, for example, in US Patent No. 6,284,471 and US Patent No. 6,277,969, which are incorporated herein by reference. StemCell Technologies SARL, 29 Chemin du Vieux Chêne, Z.I.R.S.T. 38240 Meylan, France also provide a rapid 4-step immunoassay employing the "sandwich" ELISA technique to quantitatively detect human TNFα in biological fluids. This assay utilizes a monoclonal antibody and a polyclonal rabbit antiserum raised against human recombinant TNFα for detection, and has little or no detectable cross-reactivity to human IL-1a, IL-1b, TNF-b, IL-3, IL-4, IL-6, IL-7, IL-8, G-CSF or GM-CSF. The TNFα ELISA is not inhibited by the soluble TNFα receptors p55 and p75.

Accordingly, one embodiment of the present invention provides for the level of a TNF protein in a sample to determined by a process comprising:

(i)   contacting said sample with an antibody that binds to a TNF polypeptide under conditions sufficient for binding to occur; and

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(ii)   determining the binding.

The skilled artisan is also in a position to determine a nucleic acid probe for specifically detecting mRNA encoding a TNF protein, based upon the publicly available amino acid
5   sequences referred to herein. Such probes can be used in any of the nucleic acid-based assay formats described herein, such as, for example, PCR or hybridization.

Accordingly, one embodiment of the present invention provides for the level of mRNA encoding a TNF protein in a sample to determined by a process comprising:
10   (i)   contacting said sample with a nucleic acid probe that binds to mRNA encoding a TNF polypeptide under at least low stringency hybridization conditions sufficient for binding to occur; and
       (ii)   determining the binding.

15   It will be apparent to the skilled person that the same or a different sample may be used to determine both the level of BAFF protein or TNF protein, or alternatively the level of mRNA encoding BAFF and the level of mRNA encoding BAFF. The only requirement in this respect is that, the sample is one in which the particular protein is expressed. For this reason, blood or serum are particularly preferred, since both BAFF and TNF are
20   expressed therein. Blood-based or blood-derived samples are also preferred because of the relative ease with which they are obtained from the subject.

In one embodiment of the diagnostic/prognostic methods described herein, the biological sample or test sample or reference sample, as the case may be, is obtained previously
25   from the subject. In accordance with such an embodiment, the prognostic or diagnostic method is performed *ex vivo*.

In another embodiment, the subject diagnostic method further comprises obtaining the sample from the subject, such as, for example, by surgery or other excision method, in
30   the case of blood and blood-based samples, using a syringe .

In yet another embodiment, the subject diagnostic method further comprises processing the sample from the subject to produce a derivative or extract that comprises the analyte (eg., BAFF protein or mRNA encoding a BAFF protein, or TNF protein or mRNA encoding

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a TNF protein, or any combination thereof).

Modulation of BAFF function according to the present invention, through the inhibition or promotion of at least one functional characteristic of a mammalian BAFF protein, provides
5   an effective and selective way of inhibiting or promoting receptor-mediated functions. As BAFF is expressed on B cells, mammalian BAFF proteins provide a target for selectively interfering with aberrant B cell proliferation or aberrant BAFF-mediated effects in a mammal, such as a human, particularly a human suffering from rheumatoid arthritis or colitis.
10

Accordingly, a further aspect of the present invention provides a prophylactic method of preventing or delaying the development of a cancer in a subject suffering from an inflammatory disease and/or having anti-TNF therapy or a candidate for anti-TNF therapy comprising administering an amount of an agent which inhibits a mammalian BAFF
15   function sufficient to reduce BAFF activity in a cell of the subject.

In one embodiment, the antagonist of BAFF expression comprises nucleic acid such as, for example, an antisense nucleic acid, peptide nucleic acid (PNA), ribozyme, or small interfering RNA (siRNA), short hairpin RNA (shRNA) which is complementary, in whole or
20   in part, to a target molecule comprising a sense strand, and can hybridize with the target molecule, in particular, BAFF-encoding RNA. When introduced into a cell using suitable methods, such a nucleic acid inhibits the expression of the BAFF gene encoded by the sense strand. Antisense nucleic acid, ribozymes (eg. Cech et al.,USSN 4,987,071; Cech et al., USSN 5,116,742; Bartel and Szostak, Science 261, 1411-1418, 1993), nucleic acid
25   capable of forming a triple helix (eg. Helene, Anticancer Drug Res. 6, 569-584, 1991), PNAs (Hyrup et al., Bioorganic & Med. Chem. 4, 5-23, 1996; O'Keefe et al., Proc. Natl Acad. Sci. USA 93, 14670-14675, 1996), small interfering RNAs or short hairpin RNAs may be produced by standard techniques known to the skilled artisan, based upon the sequences disclosed herein as SEQ ID Nos: 1 and/or 3.
30

Preferably, the antisense nucleic acid, ribozyme, PNA, siRNA or shRNA comprises a sequence that is complementary to at least about 12 or 15 or 18 or 20 contiguous nucleotides of a sequence having at least about 65% identity to SEQ ID NO: 1 or 3 (ie. it is complementary to BAFF RNA) and can hybridize thereto. For example, such
35   antagonistic nucleic acid can be complementary to a target nucleic acid having a

sequence selected from the group consisting of SEQ ID NO: 1 or 3 or a portion thereof sufficient to allow hybridization. Longer molecules, comprising a sequence that is complementary to at least about 25, or 30, or 35, or 40, or 45, or 50 contiguous nucleotides of BAFF RNA are also encompassed by the present invention.

5

The term "antisense nucleic acid" shall be taken to mean DNA or RNA molecule that is complementary to at least a portion of a specific mRNA molecule (Weintraub, Scientific American 262:40, 1990) and capable of interfering with a post-transcriptional event such as mRNA translation. Antisense oligomers complementary to at least about 15 contiguous

10   nucleotides of BAFF-encoding mRNA (i.e., SEQ ID NO: 1 or 3) are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target BAFF-producing cell. The use of antisense methods is well known in the art (Marcus-Sakura, Anal. Biochem. 172: 289, 1988). Preferred antisense nucleic acid will comprise a nucleotide sequence that is complementary to at least 15

15   contiguous nucleotides of a sequence encoding the amino acid sequence set forth in any one of SEQ ID NOs: 2 or 4 or a part thereof, including any one or more of the antisense oligonucleotides set forth in the Sequence Listing.

As used herein, a "ribozyme" is a nucleic acid molecule having nuclease activity for a

20   specific nucleic acid sequence. A ribozyme specific for BAFF-encoding mRNA, for example, binds to and cleaves specific regions of the mRNA, thereby rendering it untranslatable. To achieve specificity, preferred ribozymes will comprise a nucleotide sequence that is complementary to at least about 12-15 contiguous nucleotides of a sequence encoding the amino acid sequence set forth in SEQ ID NO: 2 or 4 or a part

25   thereof.

As used herein, the terms "small interfering RNA" ('siRNA"), short hairpin RNA ("shRNA"), and "RNAi" refer to homologous double stranded RNA (dsRNA) that specifically targets a gene product, thereby resulting in a null or hypomorphic phenotype. Specifically, the

30   dsRNA comprises two short nucleotide sequences derived from the target RNA encoding BAFF and having self-complementarity such that they can anneal, and interfere with expression of a target gene, presumably at the post-transcriptional level. RNAi molecules are described by Fire et al., Nature 391, 806-811, 1998, and reviewed by Sharp, Genes & Development, 13, 139-141, 1999). As will be known to those skilled in the art, short

35   hairpin RNA ("shRNA") is similar to siRNA, however comprises a single strand of nucleic acid wherein the complementary sequences are separated an intervening hairpin loop

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such that, following introduction to a cell, it is processed by cleavage of the hairpin loop into siRNA. Accordingly, each and every embodiment described herein is equally applicable to siRNA and shRNA.

5   Preferred siRNA or shRNA molecules comprise a nucleotide sequence that is identical to about 19-21 contiguous nucleotides of the target mRNA. Preferably, the target sequence in BAFF mRNA commences with the dinucleotide AA, comprises a GC-content of about 30-70% (preferably, 30-65%, more preferably 40-65% and more preferably about 45%-55%), and does not have a high percentage identity to any nucleotide sequence other 10   than a BAFF gene in the genome of the animal in which it is to be introduced, e.g., as determined by standard BLAST search.

The siRNA or shRNA is preferably capable of down regulating expression of human BAFF in a cell. In view of the high percentage conservation between murine, rat and human 15   BAFF-encoding genes, especially in the coding regions, this should not be taken to indicate a requirement for the siRNA or shRNA to be specific for human BAFF-encoding genes. In cell-based and animal models of autoimmune diseases, especially SLE or RA, it is possible and appropriate in certain circumstances for the siRNA or shRNA molecules to reduce expression of both endogenous murine BAFF, as well as ectopically expressed 20   human BAFF in a cell. Confirmation of a specific activity of an antagonist against human BAFF is preferably determined by assessing the activity of an inhibitor in a cell derived from a BAFF$^{-/-}$ mouse that has been engineered to express human BAFF.

As exemplified herein, preferred siRNA against a BAFF encoding gene comprises a 21-25   nucleotide sequence set forth in any one of SEQ ID Nos: 9-140.

The odd-numbered SEQ ID Nos. amongst SEQ ID Nos: 9-140 each comprise (i) a 19-nucleotide sequence corresponding to a human BAFF mRNA target sequence adjacent and downstream of a dinucleotide AA in said mRNA target; and (ii) a 3'-extension 30   dinucleotide TT. Each consecutive even-numbered SEQ ID No. amongst SEQ ID Nos: 9-140 each comprises (i) a 19-nucleotide sequence complementary to a human BAFF mRNA target sequence contained within the preceding odd-numbered SEQ ID No.; and (ii) a 3'-extension dinucleotide TT.

35   For producing shRNA from the exemplified siRNAs set forth in SEQ ID NOs: 9-140, the consecutive paired sense and antisense strand oligonucleotides, e.g., SEQ ID Nos: 9 and

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10, are positioned such that they flank an intervening loop sequence. Preferred loop sequences are selected from the group consisting of:

(i)   CCC (SEQ ID NO: 141);

(ii)   TTCG (SEQ ID NO: 142);

5   (iii)   CCACC (SEQ ID NO: 143);

(iv)   CTCGAG (SEQ ID NO: 144);

(v)   AAGCTT (SEQ ID NO: 145);

(vi)   CCACACC (SEQ ID NO: 146); and

(vii)   TTCAAGAGA (SEQ ID NO: 147).

10

Of these loop sequences, the sequence set forth in SEQ ID NO: 146 is particularly preferred for modulating human BAFF expression in a cell, tissue (eg., cancer cell or animal model of cancer, including a BAFF knock-out mouse expressing a non-endogenous BAFF mRNA and/or protein).

15

Preferred siRNA molecules that are selectively active against human BAFF expression compared to murine BAFF expression are derived from the sequence of the 5'-non-coding and/or 3'-non-coding region of the human BAFF gene or mRNA encoding a BAFF unique peptide.

20

The antisense RNA, ribozyme, siRNA or shRNA can be introduced directly to a cell or cell-free extract capable of expressing BAFF as naked DNA. Alternatively, DNA encoding a nucleic acid inhibitory molecule can be introduced into a cell in operable connection with a suitable promoter and transcription terminator sequence to facilitate expression of the

25 inhibitory nucleic acid.   Preferred promoters for expression in mammalian cells that express BAFF include the CMV promoter, ubiquitin promoter, U6 small nuclear RNA promoter (Lee *et al., Nature Biotech. 20*, 500-505, 2002; Miyagishi *et a;., Nature Biotech 20*, 497-500, 2002; Paul *et al., Nature Biotech. 20*, 505-508, 2002; and Yu *et al., Proc. Natl Acad. Sci USA 99*, 6047-6052, 2002), H1-RNA promoter (Brummelkamp *et al.,*

30 *Science 296*, 550-553, 2002), or other RNA polymerase III promoter. The pol III terminator is also preferred for such applications. Other promoters and terminators are not to be excluded.

In one embodiment, the DNA encoding the inhibitory nucleic acid is operably connected

35 to promoter and terminator regulatory sequences by cloning into a suitable vector that comprises the necessary promoter and transcriptional terminator sequences, and the

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recombinant vector is then introduced to the cell, tissue or organ by transient transfection of plasmid DNA, by establishing permanent cell lines or in infection with retroviral expression vectors (Barton *et al., Proc. Natl Acad. Sci USA 99,* 14943-14945, 2002; Devroe *et al., BMC Biotech. 2,* p15, 2002).

5

In a preferred embodiment, the modulatory nucleic acids are validated or tested using high throughput primary assays. It is preferred to use an *in vitro* cell-free system or cell-based system in which BAFF expression/activity is assayed for such purposes. Several vectors are known for this purpose, including, for example, the pSilencer series of vectors
10   (pSilencer 2.0, pSilencer 2.1, pSilencer 3.0, pSilencer 3.1, pSilencer 1.0-U6) provided by Ambion.

Preferred retroviral vectors, suitable for transiently transfecting into isolated cells e.g., by calcium phosphate precipitation (Ketteler *et al., Gene Ther. 9,* 477-487, 2002) in high
15   throughput screens, or for the production of transducing supernatants (Ketteler *et al., Gene Ther. 9,* 477-487, 2002) for lower-throughput screening or validation of primary screen results, include pBABE (Morgenstern *et al., Nuc. Acids Res. 18,* 3587-3596, 1990) and JZenNeo.

20   The pBabe retroviral vector constructs transmit inserted genes at high titres and express them from the Mo MuLV Long Terminal Repeat (LTR). The pBabe vectors comprise one of four different dominantly-acting selectable markers, allowing the growth of infected mammalian cells in the presence of G418, hygromycin B, bleomycin/phleomycin or puromycin. The high titre ecotropic helper free packaging cell line, omega E, reduces the
25   risk of generation of wild type Mo MuLV via homologous recombination events. Together, the pBabe vectors and omega E cell line provide high frequency gene transfer, and/or concomitant expression of BAFF with one or more other genes in a single cell (e.g., a BAFF ligand), with minimal risk of helper virus contamination.

30   For lower throughput primary screening or validation of nucleic acid antagonists, the adenoviral vectors pAdTrack and pAdTrack-CMV (He *et al., Proc. Natl Acad. Sci USA 95,* 2509-2514, 1998; pAdTrack-HP (Zhao *et al., Gene 316,* 137-141, 2003), an Ad5CMV-based vector e.g., Ad5CMV-GFP (Suoka *et al., Am. J. Respir. Cell Mol. Biol. 23,* 297-303, 2000), and pSilencer adeno 1.0-CMV (Ambion) are preferred for delivery and expression
35   in specific organs or tissues in a mouse model.

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The pAdTrack and pAdTrack-CMV vectors are particularly preferred for applications which require standardization for transfection or transduction efficiency eg., injection of adenovirus into hindlimb muscles of transgenic mouse models. The pAdTrack vector is used for production of GFP-trackable viruses containing transgenes under the control of a

5   chosen promoter. It contains the gene encoding enhanced GFP, a polylinker for insertion of exogenous transgenes surrounded by adenoviral sequences ("arms") that allow homologous recombination with pAdEasy-1. The left arm contains Ad5 nucleotides 34,931-35,935, which mediate homologous recombination with pAdEasy vectors in *E. coli*, plus inverted terminal repeat (ITR) and packaging signal sequences (nucleotides 1-480 of

10   Ad5) required for viral production in mammalian cells. The right arm contains Ad5 nucleotides 3,534-5,790, which mediate homologous recombination with pAdEasy vectors. Artificially created *PacI* sites surround both arms. The AdTrack plasmid also contains a kanamycin resistance gene from pZero 2.1 (Invitrogen) and the origin of replication from pBR322 (Life Technologies). The relatively low copy number of plasmids

15   generated with this origin is essential for the stability of large constructs in *E. coli*. The pAdTrack-CMV vector is identical to pAdTrack except for the addition of a cytomegalovirus (CMV) promoter and polyadenylation site (both from pEGFP-C1, Clontech). A polylinker is present between the CMV promoter and polyadenylation site.

20   As will be known to the skilled artisan, such adenoviral vectors are also suitable for transfection of cell lines.

In a preferred embodiment, a construct comprising an antisense nucleic acid, ribozyme, PNA, siRNA or shRNA is introduced into a suitable cell to inhibit BAFF expression and/or

25   activity therein. Such a cell provides a valuable control cell, for instance in assessing the specificity of the BAFF-ligand interaction with the parent cell or other related cell types. In another embodiment, such a construct is introduced into some or all of the cells of a mammal. The antisense nucleic acid, ribozyme, PNA, or interfering RNA, inhibits BAFF expression, and any cancer or hyperproliferative process mediated by BAFF in the cells

30   containing the construct are inhibited. Thus, a cancer or other hyperproliferative disease or condition can be treated using an antisense nucleic acid, ribozyme, PNA, siRNA or shRNA of the present invention.

In an alternative embodiment, an antagonist of BAFF activity is an antibody molecule,

35   such as, for example, a polyclonal antibody or monoclonal antibody directed against a functional epitope of the protein. Antibodies that can inhibit one or more functions

characteristic of a BAFF protein, such as a binding activity, a signalling activity, and/or stimulation of a cellular response, are clearly encompassed by the present invention. In one embodiment, antibodies of the present invention inhibit binding of a ligand (i.e., one or more ligands) to a mammalian BAFF protein and/or inhibit one or more functions
5   mediated by a mammalian BAFF protein in response to ligand binding.

The "antibodies" contemplated herein are immunoreactive with BAFF polypeptides or functional fragments thereof. Antibodies that consist essentially of pooled monoclonal antibodies with different epitope specificities, as well as distinct monoclonal antibody
10   preparations are contemplated. Monoclonal antibodies are produced from fragments of the BAFF protein that comprise one or more B cell epitopes by methods well known to those skilled in the art (Kohler et al, Nature 256:495, 1975). The term "antibody" as used herein includes intact molecules as well as fragments thereof, such as Fab and F(ab')$_2$, Fv and single chain antibody fragments capable of binding an epitopic determinant of
15   BAFF.

An "Fab fragment" consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a
20   heavy chain.

An "Fab' fragment" of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody
25   molecule treated in this manner.

An "F(ab')$_2$ fragment" of an antibody consists of a dimer of two Fab' fragments held together by two disulfide bonds, and is obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A (Fab')2 fragment.
30
An "Fv fragment" is a genetically engineered fragment containing the variable region of a light chain and the variable region of a heavy chain expressed as two chains.

A "single chain antibody" (SCA) is a genetically engineered single chain molecule
35   containing the variable region of a light chain and the variable region of a heavy chain, linked by a suitable, flexible polypeptide linker.

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Anti-BAFF antibodies or antibody fragments are generated using the entire BAFF polypeptide or an immunogenic fragment thereof (alone or linked to a suitable carrier or hapten) to immunize a subject (e.g., a mammal including, but not limited to a rabbit, goat, 5 mouse or other mammal). For example, the methods described in U.S. Pat. Nos. 5,422,110; 5,837,268; 5,708,155; 5,723,129; and 5,849,531, can be used and are incorporated herein by reference. In a preferred embodiment, the mammal being immunized does not contain endogenous BAFF (e.g., a BAFF-deficient genetically modified animal). The immunogenic preparation can further include an adjuvant, such as 10 Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic proteolytic or synthetic BAFF peptide preparation induces a polyclonal anti-BAFF antibody response.

As with all immunogenic compositions for eliciting antibodies, the immunogenically 15 effective amounts of the immunizing peptides are determined empirically. Factors to be considered include the immunogenicity of the native polypeptide, whether or not the polypeptide will be complexed with or covalently attached to an adjuvant or carrier protein or other carrier, the route of administration for the composition, i.e., intravenous, intramuscular, subcutaneous, etc., and the number of immunizing doses to be 20 administered. Such factors are known in the vaccine art and it is well within the skill of immunologists to make such determinations without undue experimentation.

The anti-BAFF antibody titer in the immunized subject is generally monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using 25 immobilized BAFF. Subsequently, the sera from the immunized subjects are tested for BAFF inhibitory activity.

Alternatively, it is also possible to immunize the subject with nucleic acid expressing BAFF using DNA immunization technology, such as that disclosed in U.S. Pat. No. 30 5,795,872 to Ricigliano et al., or alternatively, in U.S. Pat. No. 5,643,578 to Robinson et al.

In another embodiment, an antagonist of BAFF activity comprises a peptide, protein or polypeptide, including a dominant-negative mutant of a BAFF polypeptide.

35

Preferred peptidyl BAFF inhibitors are chemically or recombinantly synthesized as

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oligopeptides (approximately 10-25 amino acids in length) spanning the BAFF protein sequence (SEQ ID NO: 2 or 4). Alternatively, BAFF fragments are produced by digestion of native or recombinantly produced BAFF by, for example, using a protease, e.g., trypsin, thermolysin, chymotrypsin, or pepsin. Computer analysis (using commercially
5   available software, e.g. MacVector, Omega, PCGene, Molecular Simulation, Inc.) is used to identify proteolytic cleavage sites. The proteolytic or synthetic fragments can comprise as many amino acid residues as are necessary to partially or completely inhibit BAFF function. Preferred fragments will comprise at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length.
10

In one embodiment, peptides are selected which contain a sufficient number of B cell epitopes to elicit antibodies when administered to a mammal. Such peptides are identified by immunizing a mammal with the peptide alone, or in combination with an adjuvant, or linked to an adjuvant (e.g., a hapten). Sera from the immunized animal are tested for anti-
15   BAFF antibodies. Preferred peptides generate anti-BAFF antibodies that inhibit a BAFF function.

Preferred peptidyl BAFF inhibitors will also not comprise a sufficient number of T cell epitopes to induce T-cell mediated (e.g., cytokine) responses when determined using any
20   of a number of well known techniques, such as epitope prediction using algorithms (see e.g., Rothbard and Taylor EMBO J. 7: 93-100, 1988; Berzofsky, Philos Trans R. Soc. Lond. 323: 535-544, 1989; Rothbard, 1st Forum in Virology, Annals of the Pasteur Institute, pp 518-526, Dec. 1986; Rothbard and Taylor, Embo, 7: 93-100, 1988; EP 0 304 279; and Margalit et al., J. Immunol., 138: 2213-2229, 1987); or screening of peptide
25   inhibitors for human T cell stimulating activity or T cell proliferation assays (e.g. Proc. Natl. Acad. Sci USA, 86:1333, 1989).

Other preferred peptide inhibitors of BAFF are located on the surface of the BAFF proteins, e.g., hydrophilic regions, as well as regions with high antigenicity or fragments
30   with high surface probability scores can be identified using computer analysis programs well known to those of skill in the art (Hopp and Wood, (1983), Mol. Immunol., 20, 483-9, Kyte and Doolittle, (1982), J. Mol. Biol., 157, 105-32, Corrigan and Huang, (1982), Comput. Programs Biomed, 3, 163-8).

35   The term "dominant-negative mutant" refers to a BAFF polypeptide that has been mutated from its natural state and that interacts with a protein that BAFF normally

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interacts with thereby preventing endogenous native BAFF from forming the interaction.

Preferred dominant-negative mutants comprise variants of the native BAFF protein, such as, for example, substitution or deletion mutants. For example, a dominant-negative mutant may comprise one or more amino acid substitutions within the BAFF polypeptide such that, when expressed in a cell, the dominant-negative mutant protein competes with native endogenous BAFF for the cognate ligand, however has reduced or no activity. Means for producing mutated nucleic acid are well known to those skilled in the art and may be achieved readily e.g., using the Quick Change Mutagenesis kit supplied by Stratagene, La Jolla, California USA according to the manufacturer's instructions.

Dominant negative mutant proteins are produced by expression of nucleic acid encoding the mutant protein, essentially as described herein above for expression of peptides in cells.

As a consequence of administering an anti-BAFF antibody or an antagonistic nucleic acid, one or more hyperproliferative disorders is prevented from developing, or delayed, in a subject having a predisposition for the disease. As will be apparent from the preceding description, such susceptible subjects include individuals having anti-TNF therapy for the treatment of an inflammatory disease or a candidate for anti-TNF therapy, or alternatively or in addition, an aged subject or person at risk of inflammatory disease.

Inflammatory diseases or conditions, including chronic diseases, of humans or other species which are indicative of anti-BAFF therapy for preventing cancer, include, but are not limited to: inflammatory or allergic diseases and conditions, including systemic anaphylaxis or hypersensitivity responses, drug allergies (e.g., to penicillin, cephalosporins), insect sting allergies; inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis, ileitis and enteritis; vaginitis; psoriasis and inflammatory dermatoses such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria; vasculitis (e.g., necrotizing, cutaneous, and hypersensitivity vasculitis); spondyloarthropathies; scleroderma; respiratory allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, interstitial lung diseases (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis or colitis, or other autoimmune conditions); autoimmune diseases, such as arthritis (e.g., rheumatoid arthritis, psoriatic arthritis), multiple sclerosis, systemic lupus

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erythematosus, myasthenia gravis, diabetes, including diabetes mellitus and juvenile onset diabetes, glomerulonephritis and other nephritides, autoimmune thyroiditis, Behcet's disease; graft rejection (e.g., in transplantation), including allograft rejection or graft-versus-host disease; other diseases or conditions in which undesirable inflammatory

5   responses are to be inhibited can be treated, including, but not limited to, atherosclerosis, cytokine-induced toxicity, myositis (including polymyositis, dermatomyositis).

According to the method, one or more agents can be administered to the host by an appropriate route, either alone or in combination with another drug. An effective amount

10   of a nucleic acid or antibody agent having antagonist or agonist activity is administered. An effective amount is an amount sufficient to achieve the desired therapeutic or prophylactic effect, under the conditions of administration, such as an amount sufficient for inhibition or promotion of BAFF receptor function, and thereby, inhibition or promotion, respectively, of a receptor-mediated process (e.g., an inflammatory response).

15

A variety of routes of administration are possible including, but not necessarily limited to oral, dietary, topical, parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), and inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops) routes of administration, depending on the agent and disease or

20   condition to be treated. For respiratory allergic diseases such as asthma, inhalation is a preferred mode of administration.

Formulation of an agent to be administered will vary according to the route of administration selected (e.g., solution, emulsion, capsule). An appropriate composition

25   comprising the agent to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils, for instance.

30   Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers and the like (See, generally, Remington's Pharmaceutical Sciences, 17th Edition, Mack Publishing Co., Pa., 1985). For inhalation, the agent can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

Furthermore, where the agent is a protein or peptide, the agent can be administered via in vivo expression of the recombinant protein. In vivo expression can be accomplished via somatic cell expression according to suitable methods (see, e.g. U.S. Pat. No.

5   5,399,346). In this embodiment, nucleic acid encoding the protein can be incorporated into a retroviral, adenoviral or other suitable vector (preferably, a replication deficient infectious vector) for delivery, or can be introduced into a transfected or transformed host cell capable of expressing the protein for delivery. In the latter embodiment, the cells can be implanted (alone or in a barrier device), injected or otherwise introduced in an amount

10   effective to express the protein in a therapeutically effective amount.

A further aspect of the present invention provides a prophylactic method of preventing or delaying the development of a BAFF-mediated disease in a subject comprising administering an amount of an agent sufficient to agonize TNF or enhance the level of

15   TNF protein or TNF activity in a cell of the subject.

Alternatively or in addition, the present invention clearly contemplates prophylactic and/or therapeutic treatments wherein the level of BAFF expression is reduced in a subject in need thereof.

20

It will be apparent from the preceding description that a BAFF antagonist compound, such as, for example an anti-BAFF antibody, can be administered in conjunction with a TNF agonist compound to the subject in accordance with this aspect of the invention.

25   Preferably , the level of BAFF and the level of TNF are modulated simultaneously in the subject.

Conditions in which co-administration of BAFF antagonist compounds and TNF agonist compounds are indicated are preferably BAFF-mediated conditions, preferably BAFF-

30   mediated conditions in which TNF levels are suppressed in the subject.

A further aspect of the present invention provides an isolated antisense nucleic acid that antagonizes expression of a BAFF gene, wherein said nucleic acid comprises a nucleotide sequence capable of selectively hybridizing to mRNA encoded by the isolated

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nucleic acid of the invention.

A further aspect of the present invention provides an isolated nucleic acid that antagonizes expression of a BAFF gene, wherein said nucleic acid comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

The present invention is further described by the following non-limiting Examples.

Example 1

Production and phenotype of TNF-deficient BAFF transgenic (TNF$^{-/-}$.BAFF-Tg) mice

BAFF-Tg mice (Mackay *et al., J. Exp. Med. 190, 1697,* 1999) produce and secrete BAFF from their hepatocytes at high levels, detectable in blood (Figure 1a).

BAFF-Tg mice were maintained as heterozygotes for the transgene by backcrossing the animals onto C57BL/6 mice. We used BAFF-Tg mice after at least 10 backcrossing steps onto C57BL/6. At this point, BAFF-Tg mice were interbred to generate homozygous BAFF-Tg mice.

TNF-deficient mice, with a C57BL/6 genetic background, were provided by J. Sedgwick (DNAX, Palo Alto, CA; Korner, *et al., Eur. J. Immunol. 27, 2600,* 1997).

TNF$^{-/-}$ BAFF-Tg mice were established by breeding homozygous BAFF-Tg and TNF$^{-/-}$ mice and subsequently interbreeding the F$_1$ generation. Animals chosen for experimental analysis were homozygous for the mutant TNF allele and homozygous for the BAFF transgene.

The genotype of BAFF-Tg mice and TNF$^{-/-}$ BAFF-Tg was determined using PCR and Southern blot assays on genomic DNA obtained from 5-mm tail snips. TNF-α-specific primers were as follows:
sense-U: 5'-ATCCGCGACGTGGAACTGGCAGAA-3' (SEQ ID NO: 5); and
antisense-L: 5'-CTGCCCGGACTCCGCAAAGTCTAA-3' (SEQ ID NO: 6).
The optimal annealing temperature was 63°C, and extension time was 2 min.

Before further breeding, mice appearing homozygous by Southern blot were further

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subjected to test breeding by mating with C57BL/6 to confirm by PCR that the entire resulting F_1 progeny was positive for the BAFF transgene, indicating that the BAFF-Tg parent had copies of the transgene on both alleles. Littermates, wild type (wt; Tg negative/$TNF^{+/+}$), BAFF-Tg/$TNF^{+/+}$, and $TNF^{-/-}$/Tg negative, were kept and used as controls
5   in all experiments.

The presence of protein in mouse urine was measured using Multistix 10 SG reagent strips for urinalysis (Bayer, Diagnostics Division, Elkhart, IN; Mackay et al., J. Exp. Med. 190, 1697, 1999).
10

Antibodies

Rat anti-mouse BAFF monoclonal antibodies (mAbs) designated as clones 1C9, 9B11, and 5A8 were obtained after spleen and lymph node (LN) fusion from Wistar rats immunized with surface BAFF-expressing RBL-2H3 rat mast cells (CRL-2256; American
15 Type Culture Collection, Manassas, VA) obtained after transfection with full-length murine BAFF cDNA sequence inserted in a PCR-3 expression vector (Invitrogen, Carlsbad, CA), as previously detailed (Schneider, et al., J. Exp. Med. 189, 1747, 1999). Antibodies were purified on protein G-Sepharose for fast flow (Amersham Biosciences, Uppsala, Sweden). Abs 9B11 and 1C9 were labeled with 10x molar excess of EZ link sulfo-*N*-
20 hydroxysuccinimide biotin (Pierce, Rockford, IL), while incubating at room temperature for 30 min. The biotinylation reaction was stopped with 150 mM glycine. The sample was applied onto a desalting column to remove the free biotin (Amersham Pharmacia, Uppsala, Sweden).

25 A rat anti-mouse transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) antibody designated as clone 8F10 was produced after immunization of Wistar rats with rTACI protein, prepared as follows. The extracellular domain of murine TACI (aa 2–121) was amplified by RT-PCR using cDNA derived from FACS-sorted splenic B cells and the following primers:
30 Sense: 5'-CCGCTCGAGGCTATGGCATTCTGCCCCAAA-3' (SEQ ID NO: 7); and
antisense: 5'-CGCGGATCCTCAGCTACTTAGCCTCAATCCTGG-3' (SEQ ID NO: 8).
The amplified TACI fragment was subcloned into the *Xho*I and *Bam*HI sites of the expression vector pET-19b (Novagen, Madison, WI), resulting in the plasmid pET-19b-TACI containing an NH_2-$(His)_{10}$ tag. Correct TACI amplification and cloning were
35 confirmed by automated sequencing. BL21(DE3) *Escherichia coli* strain (Novagen) was transformed with pET-19b-TACI vector and cultured at 37°C to an $OD_{600\ nm}$ of 0.6.

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Expression of the protein was induced at 30°C by the addition of isopropyl β-D-thiogalactopyranoside to a final concentration of 1 mM, with continued incubation of the culture for 3 h. Cells were harvested by centrifugation, then lysed on ice for 1 h in a buffer containing 1% Triton X-100, 0.3 mg/ml lysozyme, and 1 mM PMSF. After cell debris
5   removal by centrifugation, 10 mM imidazole and 300 mM NaCl were added to the cleared supernatant, which was loaded onto a HiTrap chelating high performance column precharged with 100 mM NiSO$_4$ (Amersham Biosciences). After several washes of the column with buffer containing 10 mM imidazole, bound His-tagged TACI was eluted stepwise with elution buffer containing 50, 100, and finally 200 mM imidazole.
10

Immunizations

T-dependent antibody responses in control littermates as well as TNF$^{-/-}$ BAFF-Tg mice were tested using two different antigens (Ag), soluble nitrophenyl (NP)-coupled OVA, NP-OVA (Biosearch Technology, Novato, CA), and particulate SRBC (Institute of Medical and
15   Veterinary Sciences, Adelaide, Australia). Mice were immunized i.p. with 150 µg of NP-OVA emulsified in CFA (Sigma-Aldrich, St. Louis, MO), followed by a second immunization with the same dose of Ag in IFA, 21 days later. Animals were bled before the initial immunization and 7 days after each immunization to determine Ab responses. Another group of mice was immunized i.p. with 100 µl of a 10% SRBC suspension
20   (equivalent to 10^8-5 x 10^8 SRBC per mouse). Mice were bled on days 7 and 14 postimmunization, and hemagglutination assays were done, as previously described (Mackay, *et al.*, *Eur. J. Immunol.* 27, 2033, 1997). To test the immune responses to T-independent type 2 (TI-2) Ag, mice were immunized i.p. with 30 µg NP-coupled Ficoll (NP$_{59}$-aminoethyl carboxymethyl-Ficoll; Biosearch Technology). Approximately 100 µl of
25   blood was collected 1 day before the initial immunization and 7 and 14 days following the immunization to measure the NP-specific Ab response.

Flow cytometric analysis

Lymphocytes from LN, spleen, bone marrow, and thymus were isolated by mechanical
30   disruption of the organs, followed by 5-min incubation in RBC lysis buffer (0.156 M ammonium chloride, 0.01 M sodium bicarbonate, and 1 mM EDTA) on ice. For multicolor flow cytometric analysis, cells were incubated in the presence of fluorochrome- and biotin-conjugated mAb against CD21/CD35 (7G6), CD23 (B3B4), B220 (RA3-6B2), CD4 (L3T4), CD8 (53-6.7), CD44 (IM7), L-selectin (MEL-14), CD5 (53-7.3), Ly-6D (49-H4), CD45R
35   (B220), CD5 (Ly-1), CD1 (1B1) (BD PharMingen, San Diego, CA), and Cy5-conjugated goat anti-mouse IgM Ab (Jackson ImmunoResearch Laboratories, West Grove, PA).

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Where biotinylated Abs were used, streptavidin CyChrome, streptavidin PerCP (BD PharMingen), or streptavidin Cy5 (Jackson ImmunoResearch Laboratories) allowed detection. Staining with anti-TACI rat mAb 8F10 was detected using PE-conjugated anti-rat IgG, FcT chain specific (Jackson ImmunoResearch Laboratories), after blocking
5   nonspecific binding with 10 µg/ml human Ig. Rat IgG2a (BD PharMingen) was used as isotype control. All Abs were diluted in FACS buffer (PBS, 1% BSA, and 0.02% NaN₃). A FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ) with CellQuest software was used for data acquisition and analysis.

10   *ELISA*

The concentration of soluble BAFF in mouse serum was determined by coating 384-well ELISA plates (Nalge Nunc International, Rochester, NY) with 5 µg/ml purified rat anti-mouse BAFF (clone 5A8) in 50 mM sodium bicarbonate buffer (pH 9.6) at 4°C overnight. ELISA plates were washed twice with PBS/0.1% Tween 20 (Sigma-Aldrich) and blocked
15   with 1% BSA in PBS at 37°C for 2 h. To reduce interference from rheumatoid factor (RF) in the serum, Ig were cleared by treating serum dilutions with 0.05 vol of protein G-Sepharose (Amersham Biosciences) twice for 1 h at 4°C before pelleting the beads and collecting supernatants (precleared sera). A total of 20 µl/well serum or standard mouse rBAFF serial dilutions in ELISA buffer (PBS, 0.05% Tween, 1% BSA) was added to the
20   plates and incubated for 1 h at 37°C. The plates were washed three times before adding 10 µg/ml biotinylated anti-mouse BAFF (clone 1C9), followed by HRP-labeled streptavidin (DAKO, Glostrup, Denmark). The plates were washed four times before the enzymatic reaction was conducted using the tetramethylbenzidine substrate kit (BD PharMingen). The reaction was stopped by adding 2 M H₂SO₄, and the OD was measured at 450 nm
25   using Tecan SpectraImage microtiter plate reader (Bio-Tek Instruments, Winooski, VT). NP-specific Abs were detected by coating 384-well plates with 10 µg/ml NP₂-BSA (Biosearch Technology) in 50 mM sodium bicarbonate buffer (pH 9.6) at 4°C overnight. A total of 20 µl/well serum serial dilutions (untreated or precleared) was incubated in NP₂-BSA-coated 384-well plates, and NP-specific Abs bound to the plate were detected using
30   HRP-labeled goat anti-mouse Ig (Jackson ImmunoResearch Laboratories). The incubation and washing steps as well as the enzymatic reaction were conducted, as described above. The titer (log base 2) for anti-NP Abs was defined as the serum dilution giving an OD 3 times higher than that of background (1 = 1/100 dilution). ELISA for the detection of RF and anti-dsDNA and anti-ssDNA autoantibodies were done as previously
35   detailed (Mackay *et al., J. Exp. Med. 190*, 1697, 1999). The titer (log base 2) for RF and anti-ds/ssDNA autoantibody response is defined as the serum dilution giving an OD 3

times higher than that of background (1 = 1/100 dilution for RF; 1 = 1/50 dilution for anti-DNA autoantibody). Anti-NP Abs produced by animals immunized with NP-Ficoll were detected in 384-well ELISA plate coated with 10 μg/ml NP_{23}-BSA (Biosearch Technologies). Serial dilutions of the serum were added to the plate. Anti-NP Abs were detected using 1 μg/ml alkaline phosphatase-conjugated goat anti-mouse Ig (H + L), IgM, IgG1, IgG2a, IgG3, or IgA (Southern Biotechnology Associates, Birmingham, AL). Coating, incubation, and washing steps were conducted, as described above, and P-NP phosphate substrate (Sigma-Aldrich) was used for visualization. The titer (log base 2) is defined as the serum dilution giving an OD 3 times higher than that of background (where 1 = 1/100 dilution).

In vitro activation assay

Splenocytes were obtained from wild type (wt), BAFF-Tg, $TNF^{-/-}$, and $TNF^{-/-}$ BAFF-Tg (8–12 wk old) and prepared, as described above, for flow cytometry. Lymphocytes (2 x 10^6/ml) were then stimulated with 5 μg/ml goat anti-mouse μ-chain Ab (Jackson ImmunoResearch Laboratories) in complete RPMI culture medium. Cultured cells were collected at 8, 24, and 48 h for FACS analysis.

Immunohistochemistry

Various tissues (lymphoid tissues, kidneys, salivary glands, liver, lung, gut, and tumors) were collected from C57BL/6, $TNF^{-/-}$, BAFF-Tg, and $TNF^{-/-}$ BAFF-Tg mice. Tissues were either frozen in OCT compound (Tissue-Tek; Sakura, Finetek, CA) or fixed in 10% buffered Formalin and embedded in paraffin. Tissue sections, 5 μm thick, were stained with H&E for histologic examination or used for immunohistochemical staining, as previously described (Groom, *et al., J. Clin. Invest.* **109**, 59, 2002).

Results

Splenomegaly in $TNF^{-/-}$ BAFF-Tg mice is attributable to the expansion of the T2, MZ B cell, and CD4 T cell compartments

Both BAFF-Tg mice and $TNF^{-/-}$ BAFF-Tg mice had high BAFF levels in serum (Figure 1a). The splenic weight in $TNF^{-/-}$ BAFF-Tg and control littermates (wild type, BAFF-Tg, and $TNF^{-/-}$ mice), measured at ages ranging from 2 to 13 months (Figure 1b), revealed splenomegaly in $TNF^{-/-}$ BAFF-Tg mice in all age groups. Splenomegaly was not as severe as in BAFF-Tg mice, yet was significantly greater than that of wild type and $TNF^{-/-}$ littermates. The spleens of $TNF^{-/-}$ mice were consistently smaller than spleens from wild type mice (Figure 1b).

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Analysis of the T cell compartments in TNF$^{-/-}$ BAFF-Tg mice revealed a clear disproportion between CD4 and CD8 T cells (Figure 1c). The absolute numbers of CD4 T cells in 12-month TNF$^{-/-}$ BAFF-Tg mice were considerably greater than in age-matched wild type littermates (Figure 1c), whereas CD4 T cell numbers in BAFF-Tg and TNF$^{-/-}$ mice were normal, as previously described (Mackay *et al.*, 1999. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J. Exp. Med. 190:1697.*;Korner *et al*, 1997, Distinct roles for lymphotoxin-α and tumor necrosis factor in organogenesis and spatial organization of lymphoid tissue, *Eur, J, Immunol, 27:2600*; Figure 1c). In contrast, CD8 T cell numbers were slightly, but significantly fewer in TNF$^{-/-}$ BAFF-Tg mice than in control mice (Figure 1c), and this reduction was already significant in 6-month animals (data not shown).

The B220$^+$ B cell compartment was less expanded in TNF$^{-/-}$ BAFF-Tg mice when compared with littermate BAFF-Tg mice (Figure 1d, B220-positive cells), and this was not explicable by a reduced production of BAFF in TNF$^{-/-}$ BAFF-Tg mice (Figure 1a). However, a similar 4-fold increase in B cell numbers was observed in BAFF-Tg vs wild type littermates, and in TNF$^{-/-}$ BAFF-Tg vs TNF$^{-/-}$ littermates (Figure 1d). The B cell compartment of TNF$^{-/-}$ mice was significantly smaller than in wild type littermates and was reflected by reduced numbers of T1, T2, and mature B cells, but not MZ B cells (Figure 1d).

Analysis of all B cell subsets in TNF$^{-/-}$ BAFF-Tg mice revealed differences compared with BAFF-Tg mice (Figure 1d). The absolute numbers of T2 and MZ B cells, but not mature B cells, were significantly higher in TNF$^{-/-}$ BAFF-Tg mice compared with wild type littermates (Figure 1d). In contrast to previous studies (Mackay *et al*, 1999, *J, Exp, Med, 190:1697*; Groom *et al.*, Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome, *J, Clin, Invest, 109:59*; Batten *et al.*, BAFF mediates survival of peripheral immature B lymphocytes, *J, Exp, Med, 192:1453*), BAFF-Tg mice in the present experiments were homozygous for the BAFF transgene. Further to studies with heterozygous BAFF-Tg mice (Batten *et al., J, Exp, Med, 192:1453*), homozygous BAFF-Tg mice had significantly more T1 and B-1 B cells in the spleen than did wild type littermates (Figure 1d). The numbers of B-1 cells were increased in older heterozygous BAFF-Tg mice (Groom *et al., J, Clin, Invest, 109:59*) and, most likely, a dosage effect from the BAFF transgene may have accelerated this phenomenon in homozygous BAFF-Tg mice. However, B-1 B cells still represent a small proportion of all B cells in the spleen

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of homozygous BAFF-Tg mice used in these experiments (Figure 1d). The expansion of the T1 B cell compartment in homozygous BAFF-Tg mice was unclear. However, due to variations, no significant or consistent expansion of the T1 and B-1 B cell compartments was observed in the spleen of TNF$^{-/-}$ BAFF-Tg mice homozygous for the BAFF transgene

5   (Figure 1d), and similar inconclusive results were obtained analyzing B-1 B cells in peritoneal lavages (data not shown). Interestingly, there were fewer mature B cells in the spleens of TNF$^{-/-}$ mice, perhaps reflecting the absence of B cell proliferation generated within the small residual GCs that are usually seen in wild type mice under normal housing conditions, but are lacking in TNF$^{-/-}$ mice (Figure 1d). The number of plasma cells

10 in all groups of mice was variable, but appeared higher in both BAFF-Tg and TNF$^{-/-}$ BAFF-Tg mice when compared with wild type or TNF$^{-/-}$ littermates (Figure 1d).

In conclusion, splenomegaly was less pronounced in BAFF-Tg mice lacking TNF, yet was significant and associated with the selective expansion of the T2, MZ B cell, and CD4 T

15 cell compartments, but not that of mature B cells.

Impaired T-dependent, but increased T-independent immune response in TNF$^{-/-}$ BAFF-Tg mice

TNF$^{-/-}$ mice lack several important splenic features (Matsumoto *et al.*, Role of lymphotoxin

20 and the type I TNF receptor in the formation of germinal centers, *Science 271:1289*), in particular, B cell follicles and a clear T and B cell demarcation, which is also true for LN and Peyer's patches. These mice also lack FDC networks, mucosal addressin cell adhesion molecule-1 (MADCAM-1) expression on endothelial cells of MZ sinuses and on FDC, and GC formation is impaired (Matsumoto *et al.*, *Science 271:1289*). Over

25 expression of BAFF in TNF$^{-/-}$ BAFF-Tg mice did not alter the splenic, LN, and Peyer's patch defects seen in TNF$^{-/-}$ (data not shown).

T-dependent immune responses are impaired in TNF$^{-/-}$ mice (Pasparakis *et al.*, 1996, Immune and inflammatory responses in TNFα-deficient mice: a critical requirement for

30 TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response, *J, Exp, Med, 184:1397*). As BAFF is a potent stimulator of B cell activation and Ab production (Do *et al.*, 2000, Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response, *J, Exp, Med, 192:953*; Schneider *et al.*, 1999, BAFF, a novel

35 ligand of the tumor necrosis factor (TNF) family, stimulates B-cell growth, *J, Exp, Med, 189:1747*), we tested whether immune responses in TNF$^{-/-}$ BAFF-Tg mice were more

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effective than in TNF$^{-/-}$ mice. TNF$^{-/-}$ BAFF-Tg mice and control wild type, BAFF-Tg, and TNF$^{-/-}$ littermates were immunized with SRBC, a T-dependent and particulate Ag. SRBC-specific IgG responses, measured 14 days after immunization (Figure 2a), were higher in BAFF-Tg mice compared with wild type animals (Figure 2a). The initial IgM response on

5   day 7 was lower in both TNF$^{-/-}$ BAFF-Tg and TNF$^{-/-}$ mice when compared with wild type littermates (Figure 2a), and SRBC-specific IgM were no longer detected in these mice at day 14. This result indicated that the initial production of plasma cells producing SRBC-specific IgM was not sustained in TNF$^{-/-}$ BAFF-Tg mice similar to TNF$^{-/-}$ littermates. The IgG response to SRBC was similarly impaired in both TNF$^{-/-}$ and TNF$^{-/-}$ BAFF-Tg mice

10   (Figure 2a). Thus, BAFF overexpression did not improve the antibody response to SRBC in TNF$^{-/-}$ BAFF-Tg mice compared with the response detected in TNF$^{-/-}$ mice. Similar results were obtained when mice were immunized with the soluble Ag NP-OVA with adjuvant, and no difference in anti-NP antibody response was noted between TNF$^{-/-}$ BAFF-Tg mice and TNF$^{-/-}$ control littermates (Figure 2b). These results indicate that despite

15   being a strong stimulator of B cell activation and antibody production (Do *et al.*, 2000, *J, Exp, Med, 192:953*; Schneider *et al.*, 1999, *J, Exp, Med, 189:1747*), BAFF was unable to stimulate T-dependent antibody responses when overexpressed in TNF$^{-/-}$ mice.

Trinitrophenyl (TNP)-specific IgM response following immunization with TI-2 Ag TNP-Ficoll has been shown to be normal in TNF$^{-/-}$ mice, while the TNP-specific IgG responses were

20   normal at day 7 and slightly elevated after 14 days (Pasparakis, *et al.*, 1996, Immune and inflammatory responses in TNFα-deficient mice: a critical requirement for TNFα in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response, *J, Exp, Med, 184:1397*). Interestingly, TI-2 antibody response to NP-Ficoll is induced in both BAFF-Tg and TNF$^{-/-}$

25   BAFF-Tg (Figure 2c). NP-specific IgA responses were particularly increased, but also total Ig (Figure 2c) and IgG2a (data not shown). Interestingly, NP-specific IgG3 responses appeared significantly higher in TNF$^{-/-}$ BAFF-Tg mice when compared with BAFF-Tg, TNF$^{-/-}$, and wild type controls. NP-specific IgM responses appeared slightly reduced in TNF$^{-/-}$

30   mice compared with the other three mouse groups, which showed similar IgM response to NP-Ficoll.

High levels of autoantibodies in TNF$^{-/-}$ BAFF-Tg mice
We tested whether BAFF-induced emergence of autoreactive B cells in BAFF-Tg mice

35   requires TNF expression by measuring levels of autoantibodies in the serum of TNF$^{-/-}$ BAFF-Tg mice and control wild type, TNF$^{-/-}$, and BAFF-Tg littermates. Production of RF at

12 months of age was slightly higher in TNF$^{-/-}$ BAFF-Tg than in BAFF-Tg mice, and a similar observation was made for anti-dsDNA autoantibodies (p < 0.05, Figure 3a). In contrast, anti-ssDNA autoantibody responses appeared slightly higher in BAFF-Tg mice when compared with TNF$^{-/-}$ BAFF-Tg mice, yet both groups had significantly more anti-ssDNA autoantibodies than wild type and TNF$^{-/-}$, respectively (p < 0.01). Therefore, autoantibody production in BAFF-Tg mice does not require TNF and can be driven by mechanisms independent of conventional T-dependent GC immune responses.

Glomerulonephritis and features of SS in TNF$^{-/-}$ BAFF-Tg mice

BAFF-Tg mice reaching one year of age develop glomerulonephritis (Mackay *et al*, 1999, *J, Exp, Med, 190:1697*). To determine whether TNF deficiency protected against disease, we examined kidneys from TNF$^{-/-}$ BAFF-Tg mice and control mice for signs of kidney disease (Figure 3b). At 12 months of age, the kidneys of BAFF-Tg mice had advanced tissue damage, with abnormal glomeruli showing clear signs of global mesangial proliferation and obliteration of capillary lumina (Figure 3b). Kidneys of 12-month-old TNF$^{-/-}$ BAFF-Tg mice showed similar abnormalities as in BAFF-Tg mice (Figure 3b). We also detected Ig deposits in the glomeruli of both BAFF-Tg and TNF$^{-/-}$ BAFF-Tg by immunofluorescent staining with FITC-labeled anti-mouse Ig antibodies (data not shown). Therefore, progression of nephritis in BAFF-Tg mice is independent of TNF. SS-like features were also detected in BAFF-Tg mice (Groom *et al., J, Clin, Invest, 109:59*), and were characterized by acinar cell atrophy in structures lining ducts. Salivary ducts were abnormal in the salivary glands of both BAFF-Tg and TNF$^{-/-}$ BAFF-Tg mice (Figure 3c).

Lymphomas in TNF$^{-/-}$ BAFF-Tg mice

BAFF is a factor that promotes survival of B lymphoma cells (Briones *et al.*, 2002, BLyS and BLyS receptor expression in non-Hodgkin's lymphoma, *Exp, Hematol, 30:135;* Novak *et al.,* Aberrant expression of B-lymphocyte stimulator by chronic lymphocytic leukemia cells: a mechanism for survival, *Blood 100:297*). Moreover, we previously showed that the occasional aged BAFF-Tg mouse developed lymphoid masses due to B cell hyperplasia (Groom *et al., J, Clin, Invest, 109:59*). However, tumor-like masses in BAFF-Tg mice were a rare event, 0.03% in heterozygous BAFF-Tg mice (data not shown) and 2.9% in homozygous BAFF-Tg mice (Table 1). In contrast, >35% of TNF$^{-/-}$ BAFF-Tg mice at 12 months of age had developed very large lymphoid masses at various locations, most frequently in cervical LN, mesenteric LN (MLN), inguinal LN, and the small intestine (Table 1 and Figures 4, a-c, 5). Figure 4b shows a cervical lymphoid mass from a TNF$^{-/-}$ BAFF-Tg mouse, compared with the usual enlarged cervical LN found in a BAFF-Tg mouse. We

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also observed a lesion of the small intestine in TNF$^{-/-}$ BAFF-Tg mice (Figure 4c) that distended the intestinal wall, and histological analysis showed a complete invasion and destruction of the mucosa by lymphoid cells (Figure 4d), predominantly by B220$^+$ cells (data not shown). These features were those of an invasive extranodal mucosal-
5 associated lymphoid tissue-like lymphoma, associated with lymphoepithelial lesions (Maes *et al.*, Marginal zone cell lymphoma: an update on recent advances, *Histopathology 40:117*).

Histological analysis of an enlarged MLN found in TNF$^{-/-}$ BAFF-Tg mice also showed
10 abnormal features (Figure 4e). In contrast to normal MLN, MLN masses found in TNF$^{-/-}$ BAFF-Tg mice had no recognizable lymphoid structure, and immunohistochemical staining showed that the lymphoid population in these MLN masses was dominated by B220$^+$ cells (data not shown). Higher power microscopic observation of the cells in these MLN masses also revealed abnormal features (Figure 4e). Although normal lymphocytes
15 in wild type MLN were small cells characterized by a very dark nucleus and minimal cytoplasm, cells in the MLN masses collected from TNF$^{-/-}$ BAFF-Tg mice displayed neoplastic features such as a medium size with round and irregular nucleus, clumped chromatin, presence of a small nucleolus, and increased cytoplasm size often with a villous appearance. Similar observations were made with tissues from enlarged cervical
20 LN (data not shown). These pathologic features indicated intense mitotic activity and are reminiscent of neoplastic cells in MZ cell lymphomas in humans (Maes *et al.*, *Histopathology 40:117*; Franco *et al.*, Splenic marginal zone lymphoma, *Blood 101:2464*). Clonality of lymphoma cells is another feature often tested, which will require extensive cloning, sequencing of V regions of Ig genes from tumor cells, as well as Southern blot
25 experiments. Clonality, however, is thought to be less reliable than morphological changes of cells for the diagnosis of such lymphomas (Calvert *et al.*, The significance of B-cell clonality in gastric lymphoid infiltrates, *J, Pathol, 180:26*).

Strikingly, a large number of TNF$^{-/-}$.BAFF-Tg mice also developed hyperplasia of
30 individual lymph nodes that was at least 5 times greater than what was seen with the adenopathy problem in BAFF-Tg mice (see Table 1 and Figure 4). These masses contained a mixed lymphocyte population that, in many cases, reflected the lymphocyte distribution in BAFF-Tg mice (Figures 5a, 5b), however, as stated supra, these masses displayed disrupted microarchitecture, with no resemblance with normal lymphoid tissues.

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TACI expression on activated B cells from TNF$^{-/-}$ BAFF-Tg mice

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TACI, one of the BAFF receptors, has been shown to be a negative regulator of B cell growth and activation (Von Bulow *et al.*, 2001, Regulation of the T-independent humoral response by TACI, *Immunity 14:573*; Yan *et al.*, 2001, Activation and accumulation of B cells in TACI-deficient mice, *Nat, Immun, 2:638*). In addition, TACI-deficient mice develop
5 lymphomas (Seshasayee *et al.*, 2003, Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor, *Immunity 18:279*). Therefore, we examined whether the high incidence of lymphoma we see in TNF-deificient BAFF-Tg (i.e., TNF$^{-/-}$.BAFF-Tg) mice could be associated with defective TACI expression on B cells in these mice. TACI expression has been shown to be up-regulated
10 following B cell activation (Seshasayee *et al.*, 2003, *Immunity 18:279*). We generated a mouse anti-TACI mAb and tested up-regulation of TACI expression on B cells from wild type, TNF$^{-/-}$, BAFF-Tg, and TNF$^{-/-}$ BAFF-Tg littermates. Splenocytes from these mice were cultured and activated with goat anti-μ Abs for up to 48 h. At various time points, cells were harvested and stained with anti-TACI Ab, and TACI expression was analyzed by flow
15 cytometry on gated B220$^+$ cells. As described by Seshasayee *et al.*, 2003, *Immunity 18:279*, B cell activation augmented TACI expression on wild type B cells, but also on TNF$^{-/-}$ and BAFF-Tg B cells in culture, with a maximum expression level at 24 h postactivation (Figure 5). TACI expression on resting TNF$^{-/-}$ BAFF-Tg B cells was similar to that of control B cells, but anti-μ activation only weakly up-regulated TACI expression
20 on these cells (Figure 5). We were unable to show any modulation of TACI expression with rTNF on wild type splenocytes in vitro (data not shown), suggesting that a secondary effect resulting from the combination of excess BAFF and lack of TNF resulted in the development of TNF$^{-/-}$ BAFF-Tg B cells intrinsically unable to up-regulate TACI expression upon activation in vitro. Without being bound by any theory or mode of action, we
25 postulate that a defect of TACI expression on B cells from TNF$^{-/-}$ BAFF-Tg may have contributed to the higher lymphoma incidence in these mice.

Conclusions from murine data

This study showed that TNF is clearly dispensable for the inflammatory *sequela*
30 developing within the kidneys or salivary glands of BAFF-Tg mice. We clearly show that autoimmunity in BAFF-Tg does not require conventional T-dependent immune responses and GC formation, and we conclude that critical immune tolerance checkpoints during B cell maturation in the spleen are the likely stages for the breakdown of immune tolerance. Interestingly, the mature/follicular B cell compartment remained normal in TNF$^{-/-}$ BAFF-Tg
35 mice, in contrast to the T2 and MZ B cell compartments, which were enlarged, possibly reflecting the absence of proliferating mature GC B cells and impaired B cell proliferation

from T-dependent responses in TNF$^{-/-}$ BAFF-Tg mice, a BAFF-stimulated feature that would normally contribute to mature B cell expansion in BAFF-Tg mice (Mackay et al, 1999, *J, Exp, Med, 190:1697*).

5   TNF$^{-/-}$ BAFF-Tg mice were clearly different from *gld* mice lacking TNF, because in the latter splenomegaly was not observed and signs of disease were considerably reduced and delayed (Korner et al., *J, Exp, Med, 191:89*). The *gld* mice develop SLE-like features similar to those of BAFF-Tg mice, yet the cause is linked to inappropriate survival of both B and T cells (Siegel et al., 2000, The multifaceted role of Fas signaling in immune cell
10   homeostasis and autoimmunity, *Nat, Immun, 1:469*). *Fas* ligand is important in promoting activation-induced cell death and controlling immune responses (Siegel et al., 2000, *Nat, Immun, 1:469*); this ligand is highly expressed in germinal centers (GCs), participates in GC B cell apoptosis (Van Eijk et al., Death-receptor contribution to the germinal-center reaction, *Trends Immunol, 22:677*), and may also contribute to eliminate self-reactive
15   cells at this site. However, with an excess of BAFF, impaired T-dependent immune responses and absence of GC did not influence the manifestation of autoimmunity in the TNF$^{-/-}$ BAFF-Tg mice, pointing to the dysregulation of a separate pathway of B cell activation.

20   Antibody responses, in particular IgA responses, to the TI-2 Ag NP-Ficoll were higher in both BAFF-Tg and TNF$^{-/-}$ BAFF-Tg mice. Both MZ and B-1 B cells respond to T-independent antigens (Martin et al., 2000, B-cell subsets and the mature preimmune repertoire: marginal zone and B1 B cells as part of a "natural immune memory,", *Immunol, Rev, 175:70*); however, in the case of NP-Ficoll immunization, only MZ B cells,
25   but not B-1 B cells, participate in the response (Berland et al., 2002, Origins and functions of B-1 cells with notes on the role of CD5, *Annu, Rev, Immunol, 20:253*). BAFF has been shown to induce IgA switching in B cells (Litinskiy et al., 2002, DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL, *Nat, Immun, 3:822*), and elevated IgA levels are detected in the serum of BAFF-Tg mice (Khare et al.,
30   2000, Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice, *Proc, Natl, Acad, Sci, USA 97:3370*). Mucosal immune responses are normally a major source of IgA production, and both T-dependent and T-independent mechanisms have been shown to trigger IgA production in the gut (Fargarasan et al., 2003, Intestinal IgA synthesis: regulation of front-line body defenses, *Nat, Rev, Immunol, 3:63*). Because B-1
35   B cells are unresponsive to NP-Ficoll immunization, elevated numbers of MZ B cells seem to be the likely explanation for the induced response to NP-Ficoll. LPS-activated MZ B

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cells produce more IgA than mature B cells in the presence of BAFF in vitro. In addition, while NP-specific IgA may be MZ B cell derived following NP-Ficoll immunization of BAFF-Tg mice, we cannot exclude that B-1 B cells may also produce elevated levels of IgA with other specificities in these mice. Abnormal IgA production can be associated with kidney

5   pathologies such as IgA nephropathy (Donadio et al., 2002, IgA nephropathy, N, Engl, J, Med, 347:738), and IgA deposits are detected in the kidneys of BAFF-Tg mice.

The MZ B cell compartment is consistently enlarged in both BAFF-Tg and TNF^{-/-} BAFF-Tg mice. MZ B cells display some degree of poly- and self-reactivity and are suspected to

10 participate in autoimmune diseases (Martin et al., 2002, Marginal-zone B cells, Nat, Rev, Immunol, 2:323; Li et al., 2002, Autoreactive B cells in the marginal zone that express dual receptors, J, Exp, Med, 195:181; Zeng et al.,2000, A role for CD1 in the pathogenesis of lupus in NZB/NZW mice, J, Immunol, 164:5000). In addition, injection of prolactin or estrogen in mice led to lupus-like autoimmune disorders associated with the

15 expansion of the MZ B cell compartment (Grimaldi et al., 2001, Expansion and activation of a population of autoreactive marginal zone B cells in a model of estrogen-induced lupus, J, Immunol, 167:1886; Peeva et al., 2003, Prolactin modulates the naive B cell repertoire, J, Clin, Invest, 111:275). Interestingly, treatment of BAFF-Tg mice with a lymphotoxin-β-R-Ig fusion protein that neutralized lymphotoxin-α/β can specifically

20 eliminate MZ B cells and protect these mice against autoimmune kidney disease. Moreover, while MZ-like B cells are normally sessile and found solely in the spleen of wt mice, such B cells were detected in LN, blood, and salivary glands of BAFF-Tg mice (Groom et al., J, Clin, Invest, 109:59; Batten et al., J, Exp, Med, 192:1453), suggesting a potential role in autoimmune inflammation. This observation is telling, because

25 sequestration of autoreactive B cells in the MZ is essential for maintenance of immune tolerance (Li et al., 2002, Anti-DNA B cells in MRL/lpr mice show altered differentiation and editing pattern, J, Exp, Med, 196:1543). Collectively, these observations suggest that location and function, rather than size, of the MZ B cell population may be key factors determining whether these cells contribute to autoimmune mechanisms.

30

Interestingly, high numbers of activated CD4 T cells were found in TNF^{-/-} BAFF-Tg mice. Although TNF is known to be a potent mitogenic factor for activated T cells (Tartaglia et al., 1993, Stimulation of human T-cell proliferation by specific activation of the 75-kDa tumor necrosis factor receptor, J, Immunol, 151:4637), other reports have shown that TNF

35 can attenuate TCR signaling, and thereby protect locally against the emergence of autoreactive T cells (Kollias et al., 2002, Role of TNF/TNFR in autoimmunity: specific TNF

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receptor blockade may be advantageous to anti-TNF treatments, *Cytokine Growth Factor Rev, 13:315*). Increased numbers of CD4 T cells in $TNF^{-/-}$ BAFF-Tg mice may reflect the lack of this regulatory mechanism, which could explain exacerbation of disease in $TNF^{-/-}$ BAFF-Tg mice.

5

In addition, BAFF can stimulate T cell responses and production of inflammatory cytokines (Huard *et al.,* T cell costimulation by the TNF ligand BAFF, *J, Immunol, 167:6225*).

One of the most striking observations in $TNF^{-/-}$ BAFF-Tg mice was the emergence of
10   conspicuous lymphoid tumors in >35% of 1-year-old animals. These results show that BAFF-mediated autoimmune disease is not TNF-dependent. Onset of autoimmune symptoms is accelerated in $TNF^{-/-}$.BAFF-Tg mice and a high incidence of hyperplasic lymphoid masses was observed. These tumors were located mostly in the cervical LN near the salivary glands, but also in the gut, similar to extranodal MZ cell lymphomas or
15   mucosal-associated lymphoid tissue-type lymphomas. Such lymphomas are a complication in SS (Abbondanzo, 2001, Extranodal marginal-zone B-cell lymphoma of the salivary gland, *Ann, Diagn, Pathol, 5:246*), a disease associated with elevated BAFF levels (Groom *et al., J, Clin, Invest, 109:59*; Mariette *et al.,* 2003, The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjögren's syndrome, *Ann,*
20   *Rheum, Dis, 62:168*). We have previously described occasional instances of BAFF-Tg mice developing lymphoid masses and B cell hyperplasia (Groom *et al., J, Clin, Invest, 109:59*), but the actual incidence was generally quite low. However, this complication is dramatically exacerbated in $TNF^{-/-}$ BAFF-Tg mice, indicating that TNF plays a critical role in protecting BAFF-Tg mice against B cell lymphoma.

25

TACI is present on the surface of naive $TNF^{-/-}$ BAFF-Tg B cells, but its expression failed to augment upon activation in vitro. Whether this anomaly is associated with increased tumor development in $TNF^{-/-}$ BAFF-Tg mice in addition to lack of TNF-mediated tumor surveillance is not clear.

30
The observation of elevated tumor incidence in $TNF^{-/-}$ BAFF-Tg mice is relevant to clinical observations in humans, because, recently, a risk for development of lymphomas from anti-TNF therapies has emerged, with the identification of 26 cases of lymphomas in a cohort of patients treated with anti-TNF reagents (Brown *et al.,* 2002, Tumor necrosis
35   factor antagonist therapy and lymphoma development, *Arthritis Rheum, 46:3151*). These results suggest that patients with high serum BAFF are more at risk of developing

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adverse neoplastic problems with anti-TNF therapies and that TNF has a protective effect against BAFF-mediated risk of lymphoma. In this respect, it is also known that certain individuals with SS, SLE, or RA patients do express very high BAFF levels in serum, or within inflammatory lesions (Cheema *et al.,* 2001, Elevated serum B lymphocyte

5   stimulator levels in patients with systemic immune-based rheumatic diseases, *Arthritis Rheum, 44:1313*; Groom *et al., J, Clin, Invest, 109:59).*

10

TABLE 1

Hyperplasic mass incidence and distribution in 11-13 month old mice, TNF-/-.BAFF-Tg mice compared with groups of WT, TNF-/- and BAFF-Tg mice.

Genotype	No. mice dissected	Mass No.	Mass Location [a] (No. cases)	Incidence (%)
WT	20	0	None	0
TNF -/-	15	0	None	0
BAFF transgenic (BAFF-Tg)	34	1	CLN (1)	2.9
TNF -/- BAFF-Tg	63	24	CLN (11) MLN (7) ALN (1) SI (1) SI+MLN (2) CLN+MLN (1)	38.1 $p<0.0001$[b]

(a) CLN, cervical lymph node; MLN, mesenteric lymph node; ALN, axillary lymph node;

15   SI, small intestine.

(b) the p-value was calculated using the hypergeometric distribution test.

EXAMPLE 2

20   *Preparation of a monoclonal antibody that binds to human BAFF*

A monoclonal antibody that specifically binds the human BAFF polypeptide (i.e., comprising an amino acid sequence set forth in SEQ ID NO: 2) is produced using

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methods known in the art. Briefly, the human BAFF (hBAFF) polypeptide is produced by amplifying the open reading frame of SEQ ID NO: 1 and cloning the amplified DNA into a mammalian Gateway expression vector (Invitrogen) pcDNA-DEST40 (CMV/C-terminal V5-6xHis) or the *E.coli* Gateway expression vector pET-DEST42 (T7/*lac* promoter, C-
5 terminal V5-6xHis) by recombination. The expressed polypeptide is purified on nickel columns by virtue of it binding via the 6XHis tag on the recombinant protein according to the instructions provided by Invitrogen.

Female BalB/c mice are immunized with the recombinant human BAFF polypeptide.
10 Initially mice are sensitised by intraperitoneal injection of Hunter's Titermax adjuvant (CytRx Corp., Norcross, GA,). Three boosts of the peptide are administered at 2, 5.5 and 6.5 months post initial sensitization. The first of these boosts is a subcutaneous injection while the remaining are administered by intraperitoneal injection. The final boost is administered 3 days prior to fusion.
15

The splenocytes of one of the immunized BALB/c mice are fused to X63-Ag8.653 mouse myeloma cells using PEG 1500. Following exposure to the PEG 1500, cells are incubated at 37°C for 1 hour in heat inactivated foetal bovine serum. Fused cells are then transferred to RPMI 1640 medium and incubated overnight at 37°C with 10% CO_2. The
20 following day cells are plated using RPMI 1640 media that has been supplemented with macrophage culture supernatants.

Two weeks after fusion, hybridoma cells are screened for antibody production by solid phase ELISA assay. Standard microtitre plates are coated with the recombinant human
25 BAFF polypeptide in a carbonate-based buffer. Plates are then blocked with BSA, washed and then the test samples (i.e. supernatant from the fused cells) is added, in addition to control samples, (i.e. supernatant from an unfused cell). Antigen-antibody binding is detected by incubating the plates with goat-anti-mouse HRP conjugate (Jackson ImmunoResearch Laboratories) and ABTS peroxidase substrate system (Vector
30 Laboratories, Burlingame, Ca 94010, USA). Absorbance is read on an automatic plate reader at a wavelength of 405 nm.

Hybridoma cells that produce antibodies that bind the hBAFF polypeptide are selected and retained. Colonies that are identified as positive by these screens continue to be
35 grown and screened for several further weeks. Stable colonies are then isolated and stored at –80°C.

Positive stable hybridomas are then cloned by growing in culture for a short period of time and diluting the cells to a final concentration of about 0.1 cells/well of a 96 well tissue culture plate. These clones are then screened as described above. This procedure is

5   then repeated in order to ensure the purity of the clone.

Four different dilutions, 5 cells/well, 2 cells/well, 1 cell/well, 0.5 cells/well of the primary clone are prepared in 96-wells microtiter plates to start the secondary cloning. Cells are diluted in IMDM tissue culture media containing the following additives: 20% fetal bovine

10 serum (FBS), 2 mM L-glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 1% GMS-S, 0.075% NaHCO$_3$. To determine clones that secrete anti-hBAFF antibody, supernatants from individual wells of the 0.2 cells/well microtiter plate are withdrawn after two weeks of growth and tested for the presence of antibody by ELISA assay as described above.

15

All positive clones are adapted and expanded in RPMI media containing the following additives: 10% FBS, 2 mM L-glutamine, 100 units/ml of penicillin, 100 .μg/ml of streptomycin, 1% GMS-S, 0.075% NaHCO$_3$, and 0.013 mg/ml of oxaloacetic acid. A specific antibody is purified by Protein A affinity chromatography from the supernatant of

20 cell culture.

The titer of the antibodies produced using this method are determined using the Easy Titer kit available from Pierce (Rockford, II, USA). This kit utilises beads that specifically bind mouse antibodies, and following binding of such an antibody these beads aggregate

25 and no longer absorb light to the same degree as dissociated beads. Accordingly, the amount of an antibody in the supernatant of a hybridoma is assessed by comparing the OD measurement obtained from this sample to the amount detected in a standard, such as for example mouse IgG.

30

EXAMPLE 3

Immunoassays to determine hBAFF levels in human sera

35 A monoclonal antibody that binds to human BAFF is produced essentially as described in Example 2. Alternatively, a mouse anti-hBAFF monoclonal antibody produced in BALB/c

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mice using recombinant human BAFF and purified from ascites by Protein A chromatography column is available publicly from Research Diagnostics Inc., Flanders NJ 07836, USA. Alternatively, an affinity-purified goat anti-hBAFF:Biotin conjugate is also available from Research Diagnostics Inc. A wide range of other suitable anti-hBAFF
5   antibodies for use in the following assay are publicly available from Abcam, Cambridge Science Park, Cambridge, United Kingdom; Novus Biologicals, Inc., Littleton, CO 80160, USA; and PeproTech Inc., Princeton Business Park, Rocky Hill, NJ 08553, USA. Petrotech also supply recombinant hBAFF protein comprising the full-length hBAFF polypeptide or the C-terminal portion thereof. Any one of such antibodies is used in a
10 single site ELISA that detects hBAFF without prior capture of the protein.

To determine suitability of any monoclonal antibody for use in an immunoassay, the candidate monoclonal antibodies are absorbed to a microtitre plate at 20°C for 16 hours and the plates are washed and blocked for 1 hour. Recombinant hBAFF protein is
15 serially diluted, added to wells of the microtitre plate and incubated for 1 hour. Following a further wash, the candidate monoclonal antibodies, conjugated to horseradish peroxidase (HRP) using a HRP conjugation kit (Alpha Diagnostics International, Inc., San Antonio, TX, USA), are separately added to each well of the plate and incubated. Plates are then washed as before and substrate ABTS (Sigma Aldrich, Sydney, Australia) is added to
20 each well. Reactions are stopped after approximately 20 minutes and absorbance values measured at 415 nm. The amount of absorbance detected in negative control wells (no hBAFF added) is subtracted from the absorbance of each other well to determine the amount of antibody bound to the hBAFF polypeptide. Those antibodies that provide a significant signal above background are selected.

25

For two-site immunoassays, the antibodies are further screened to select at least two antibodies that bind to different epitopes on hBAFF, such as, for example, monoclonal antibodies that do not cross-react. Standard linear epitope mapping experiments are performed to demonstrate the absence of significant cross-reactivity between monoclonal
30 antibodies. Alternatively, or in addition, the antibodies are labeled using different ligands that are differentially detectable e.g., different fluorophores that emit at different wavelengths to permit high throughput wherein antibodies carrying different labels are arrayed in standard two-site ELISA tests and those antibodies that simultaneously and contemporaneously bind to hBAFF protein are selected as suitable antibody
35 combinations. The advantage of this latter approach is that there is no need to perform

linear epitope mapping.

For two-site ELISA, human sera are obtained from one or more subjects suffering from rheumatoid arthritis and receiving a recommended dosage of Etanercept, Remicade or Humira for more than about 2 weeks. Sera are diluted 1:10 (v/v) in PBS comprising 1% (w/v) BSA and pre-cleared of human Ig on protein A-Sepharose beads (10% (v/v) pelleted beads, Amersham Pharmacia Biotech) overnight at 4°C. A capture antibody (e.g. a rat anti-hBAFF antibody; 2 μg/ml) is adsorbed to the wells of a 96-well or 256-well microtitre plate (Nalge Nunc International, Rochester, NY, USA) overnight at 4°C in PBS. Plates are washed in PBS comprising 0.1% (v/v) Tween 20, and then blocked in a suitable blocking solution e.g., PBS comprising 10% casein hydrolysate. After blocking, serial dilutions of the pre-cleared sera are added, followed by a detection antibody (mouse anti-hBAFF monoclonal antibody; 0.5 μg/ml). Alkaline phosphatase-labeled streptavidin (Jackson ImmunoResearch Laboratories, Inc.) and the corresponding alkaline phosphatase substrate Sigma 104 (Sigma Chemical Co.) are used for detection. The reactions are stopped using 3N NaOH. Plates are read at an OD of 405 nm, and a standard curve is constructed using known quantities of recombinant hBAFF diluted in human serum and treated as described for patient samples. Statistical analyses are performed using StatView software (Abacus Concept Inc., USA) and ANOVA.

In another immunoassay format, For two-site ELISA, human sera are obtained from one or more subjects suffering from rheumatoid arthritis and receiving a recommended dosage of Etanercept, Remicade or Humira for more than about 2 weeks. Sera are cleared of IgG as described above, and added to a test strip that comprises a rat anti-hBAFF monoclonal antibody that has been previously conjugated to visible colloidal gold (available from structure Probe Inc., West Chester, PA, USA). BAFF protein in the sera binds to the antibody, and the sample then migrates (by capillary action) up the test strip, passing over a second monoclonal antibody, a mouse anti-hBAFF antibody, bound to the test strip in a specific localised area (i.e. a line). BAFF protein present in the sera is captured by this second antibody. A significant concentration of BAFF protein captured by the second antibody causes a visible line to form on the test strip. Accordingly, the formation of this line indicates that a sample comprises an elevated amount of BAFF protein. The amount of BAFF required to form a visible line is varied by altering the amount of the second antibody bound to the test strip.

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Data indicate that elevated BAFF levels are present in subjects receiving anti-TNF therapy compared to subjects not receiving anti-TNF therapy.

5   Subjects showing normal and elevated levels of BAFF protein in sera are monitored for the appearance of lymphoma, preferably a non-Hodgkin's lymphoma (NHL) (e.g., diffuse large B cell NHL, large cell NHL, small T cell NHL, diffuse large cell NHL, B cell NHL, follicular mixed small and large cell NHL, mantle cell B cell NHL, or small lymphocytic B cell NHL). A positive correlation exists between the appearance of malignancies and

10   elevated serum BAFF levels.

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WE CLAIM:

1.   A method for determining a cancer cell in a subject that has commenced or undergone anti-TNF therapy or a predisposition to developing cancer in a subject that is a candidate for anti-TNF therapy or has commenced or undergone anti-TNF therapy , said method comprising determining the level of expression of a BAFF gene in a sample of said subject wherein elevated expression of said gene relative to the level of expression of a BAFF gene in a normal or healthy subject that does not have elevated BAFF expression is indicative of said cancer cell or said predisposition to developing cancer, and wherein said BAFF gene comprises a nucleotide sequence selected from the group consisting of:

    (a)   a sequence encoding a polypeptide comprising an amino acid sequence having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4;

    (b)   a sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3;

    (c)   a sequence that hybridizes specifically under at least low stringency conditions to SEQ ID NO: 1 or SEQ ID NO: 3;

    (d)   a sequence comprising a protein-encoding region of (b) or (c); and

    (e)   a sequence complementary to any one of (a) to (d).

2.   The method of claim 1, wherein the cancer is a lymphoma.

3.   The method of claim 2, wherein the lymphoma is a non-Hodgkin's lymphoma (NHL).

4.   The method of claim 3 wherein the NHL is selected from the group consisting of diffuse large B cell NHL, large cell NHL, small T cell NHL, diffuse large cell NHL, B cell NHL, follicular mixed small and large cell NHL, mantle cell B cell NHL and small lymphocytic B cell NHL.

5.   The method of claim 1 wherein the cancer is a leukemia.

6.   The method of claim 1 wherein the cancer is a myeloma.

7.   The method of claim 1 wherein the cancer is a carcinoma.

8.   The method of claim 7 wherein the carcinoma is selected from the group

consisting of head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer, renal cell cancer, bladder cancer, a gynecological carcinoma, prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, epithelial vulval carcinoma and medulloblastoma.

9.   The method of claim 1, wherein the sample comprises blood or a fraction of blood.

10.   The method of claim 9 wherein the fraction of blood comprises serum or a fraction of serum.

11.   The method of claim 10 wherein the fraction of serum comprises serum cleared of IgG.

12.   The method of claim 1 wherein the fraction of blood comprises a buffy coat preparation or a cell type selected from the group consisting of peripheral blood lymphocytes, neutrophils, macrophages, T cells and dendritic cells.

13.   The method of claim 1 wherein the sample is prepared on a solid matrix.

14.   The method of claim 1 wherein the sample is solubilized.

15.   The method of claim 1 wherein the sample has been obtained previously from a subject.

16.   The method of claim 1 wherein expression of a BAFF gene is determined by a process comprising determining the level of a polypeptide encoded by the BAFF gene in a test sample from the subject.

17.   The method of claim 16 comprising:
      expression of a BAFF gene is determined by a process comprising determining the level of a polypeptide encoded by the BAFF gene in a test sample from the subject.   In accordance with this embodiment, it is preferred for the assay to comprise:
      (a)   determining the level of a polypeptide encoded by the BAFF gene in a test sample from the subject; and

(b)   comparing the level of the polypeptide determined at (a) to the level of said polypeptide in a comparable sample from a healthy or normal subject that does not have elevated BAFF expression,

wherein a level of a polypeptide at (a) that is enhanced in the test sample relative to the comparable sample from the healthy or normal subject is indicative of elevated expression of a BAFF gene.

18.   The method of claim 16 wherein the level of the polypeptide is determined by a process comprising contacting an antibody that binds specifically to a polypeptide encoded by the BAFF gene to the test sample for a time and under conditions sufficient for an antigen-antibody complex to form and then detecting the complex.

19.   The method of claim 18 comprising an immunohistochemical (IHC) detection means.

20.   The method of claim 18 comprising an enzyme-linked immunosorbent assay (ELISA).

21.   The method of claim 18 comprising a Western blot immunoassay.

22.   The method of claim 18 wherein the antibody is a polyclonal antibody.

23.   The method of claim 18 wherein the antibody is a monoclonal antibody.

24.   The method of claim 1 wherein expression of a BAFF gene is determined by a process comprising determining the level of mRNA encoded by a BAFF gene in a test sample from the subject.

25.   The method of claim 24 comprising:

(a)   determining the level of mRNA encoded by a BAFF gene in a test sample from the subject; and

(b)   comparing the level of mRNA determined at (a) to the level of mRNA encoded by a BAFF gene in a comparable sample from a healthy or normal subject that does not have elevated BAFF expression,

wherein a level of mRNA at (a) that is enhanced in the test sample relative to the comparable sample from a healthy or normal subject is indicative of elevated

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expression of a BAFF gene.

26.   The method of claim 25 wherein the mRNA is detected by contacting a nucleic acid probe to nucleic acid in the test sample for a time and under conditions sufficient for hybridization to occur and then detecting the hybridization.

27.   The method of claim 26 wherein the nucleic acid probe comprises a nucleotide sequence selected from the group consisting of:
(a)   a sequence encoding an amino acid sequence having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4;
(b)   a sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3;
(c)   a sequence that hybridizes specifically under at least low stringency conditions to SEQ ID NO: 1 or SEQ ID NO: 3;
(d)   a sequence comprising a protein-encoding region of (b) or (c);
(e)   a sequence complementary to any one of (a) to (d); and
(f)   a sequence comprising at least about 20 contiguous nucleotides of any one of (a) to (e).

28.   The method of claim 27 wherein the nucleic acid probe comprises a nucleotide sequence selected from the group consisting of:
(a)   a sequence encoding the amino acid sequence set forth in SEQ ID NO: 2;
(b)   the sequence set forth in SEQ ID NO: 1;
(c)   a sequence that hybridizes specifically under at least low stringency conditions to SEQ ID NO: 1;
(d)   a sequence comprising a protein-encoding region of (b) or (c);
(e)   a sequence complementary to any one of (a) to (d); and
(f)   a sequence selected from the group consisting of SEQ ID Nos: 9-140.

29.   The method of claim 28 wherein the nucleic acid probe comprises a nucleotide sequence selected from the group consisting of SEQ ID Nos: 9-140.

30.   The method of claim 27 wherein the nucleic acid probe is labeled with a reporter molecule and hybridization is detected by detecting the reporter molecule.

31.   The method of claim 27 wherein hybridization is detected by detecting nucleic acid

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amplified in a polymerase chain reaction (PCR).

32.   The method of claim 30 or 31 wherein the hybridization is carried out *in situ* on a test sample consisting of a histology specimen.

33.   The method of claim 30 or 31 wherein the hybridization is carried out on a nucleic acid microarray of test samples or a tissue microarray of test samples.

34.   The method of claim 30 or 31 wherein the hybridization is carried out in solution.

35.   The method of claim 1 wherein the subject is a human.

36.   The method of claim 1 wherein the subject has undergone or commenced anti-TNF therapy in combination with an immunosuppressive drug.

37.   The method of claim 36 wherein the immunosuppressive drug is selected from the group consisting of methotrexate, azathioprine and a corticosteroid.

38.   The method of claim 1 wherein the subject is infected with Epstein-Barr virus (EBV).

39.   The method of claim 1 wherein the subject suffers from an autoimmune disease or inflammatory disease.

40.   The method of claim 39 wherein the autoimmune disease or inflammatory disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis and colitis.

41.   The method of claim 40 wherein the autoimmune disease or inflammatory disease is rheumatoid arthritis.

42.   The method of claim 1 wherein the subject is an aged subject.

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43.   The method of claim 1 further comprising obtaining the sample from a subject.

44.   The method of claim 1 further comprising processing a sample from the subject to produce a derivative or extract that comprises a BAFF protein or mRNA encoding a BAFF protein.

45.   The method of claim 44 wherein the derivative or extract comprises a TNF protein or mRNA encoding a TNF protein.

46.   The method of claim 1 further comprising determining the level of expression of a TNF protein or nucleic acid encoding a TNF protein in a biological sample derived from the subject, wherein a reduced level of the TNF protein or nucleic acid encoding the TNF protein in the sample is indicative of a cancer cell in the subject or a predisposition for the cancer.

47.   Use of an antibody that binds to a BAFF protein to detect a cancer cell in a sample from a subject that has commenced or undergone anti-TNF therapy or to detect a predisposition to developing cancer in a subject that is a candidate for anti-TNF therapy or has commenced or undergone anti-TNF therapy, wherein the BAFF protein comprises an amino acid sequence having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4.

48.   The use according to claim 47 wherein the antibody is a polyclonal antibody.

49.   The use according to claim 47 wherein the antibody is a monoclonal antibody.

50.   The use according to claim 47 wherein the cancer is a lymphoma.

51.   The use according to claim 50, wherein the lymphoma is a non-Hodgkin's lymphoma (NHL).

52.   The use according to claim 51 wherein the NHL is selected from the group consisting of diffuse large B cell NHL, large cell NHL, small T cell NHL, diffuse large cell NHL, B cell NHL, follicular mixed small and large cell NHL, mantle cell B cell NHL and small lymphocytic B cell NHL.

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53.   The use according to claim 47 wherein the cancer is a leukemia.

54.   The use according to claim 47 wherein the cancer is a myeloma.

55.   The use according to claim 47 wherein the cancer is a carcinoma.

56.   The use according to claim 55 wherein the carcinoma is selected from the group consisting of head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer, renal cell cancer, bladder cancer, a gynecological carcinoma, prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, epithelial vulval carcinoma and medulloblastoma.

57.   The use according to claim 47 wherein the sample comprises blood or a fraction of blood.

58.   The use according to claim 57 wherein the fraction of blood comprises serum or a fraction of serum.

59.   The use according to claim 58 wherein the fraction of serum comprises serum cleared of IgG.

60.   The use according to claim 57 wherein the fraction of blood comprises a buffy coat preparation or a cell type selected from the group consisting of peripheral blood lymphocytes, neutrophils, macrophages, T cells and dendritic cells.

61.   The use according to claim 60 wherein the sample is prepared on a solid matrix.

62.   The use according to claim 61 wherein the sample is solubilized.

63.   The use according to claim 47 wherein the sample has been obtained previously from a subject.

64.   Use of a nucleic acid probe that hybridizes specifically to mRNA encoding a BAFF

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protein to detect a cancer cell in a sample from a subject that has commenced or undergone anti-TNF therapy or to detect a predisposition to developing cancer in a subject that is a candidate for anti-TNF therapy or has commenced or undergone anti-TNF therapy, wherein the mRNA encoding a BAFF protein comprises a nucleotide sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3.

65.   The use according to claim 64 wherein the nucleic acid probe comprises a sequence that is complementary to at least about 20 contiguous nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3.

66.   The use according to claim 64 wherein the nucleic acid probe comprises a nucleotide sequence set forth in any one of SEQ ID Nos: 9-140.

67.   The use according to claim 64 wherein the cancer is a lymphoma.

68.   The use according to claim 67, wherein the lymphoma is a non-Hodgkin's lymphoma (NHL).

69.   The use according to claim 68 wherein the NHL is selected from the group consisting of diffuse large B cell NHL, large cell NHL, small T cell NHL, diffuse large cell NHL, B cell NHL, follicular mixed small and large cell NHL, mantle cell B cell NHL and small lymphocytic B cell NHL.

70.   The use according to claim 64 wherein the cancer is a leukemia.

71.   The use according to claim 64 wherein the cancer is a myeloma.

72.   The use according to claim 64 wherein the cancer is a carcinoma.

73.   The use according to claim 72 wherein the carcinoma is selected from the group consisting of head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer, renal cell cancer, bladder cancer, a gynecological carcinoma, prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, epithelial vulval carcinoma and

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medulloblastoma.

74.   The use according to claim 64 wherein the sample comprises blood, plasma or a
       fraction of plasma.

75.   The use according to claim 74 wherein the fraction of plasma comprises a buffy
       coat preparation or a cell type selected from the group consisting of neutrophils,
       macrophages, T cells and dendritic cells.

76.   The use according to claim 64 wherein the sample is prepared on a solid matrix.

77.   The use according to claim 64 wherein the sample is solubilized.

78.   The use according to claim 64 wherein the sample has been obtained previously
       from a subject.

79.   A method of identifying a compound that reduces or antagonizes expression of a
       BAFF gene comprising:

       (a)   administering a candidate compound to a cell that expresses a BAFF gene
              at an elevated level; and

       (b)   determining the level of expression of a BAFF gene in the presence of the
              compound relative to the level of expression of the gene in the absence of
              the compound,

       wherein reduced level of expression of a BAFF gene in the presence of the
       compound indicates that the compound is an antagonist of BAFF gene expression
       and wherein said BAFF gene comprises a nucleotide sequence selected from the
       group consisting of:

       (i)   a sequence encoding a polypeptide comprising an amino acid sequence
              having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4;

       (ii)   a sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID
              NO: 3;

       (iii) a sequence that hybridizes specifically under at least low stringency
              conditions to SEQ ID NO: 1 or SEQ ID NO: 3;

       (iv)   a sequence comprising a protein-encoding region of (ii) or (iii); and

       (v)   a sequence complementary to any one of (i) to (iv).

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80.   The method of claim 79 wherein the cell is a cell that over expresses a BAFF gene by virtue of having been stably transformed or transiently transfected with a nucleic acid comprising a BAFF gene.

5   81.   The method of claim 80 wherein the cell is a 293 cell that has been stably transformed or transiently transfected with a nucleic acid comprising a BAFF gene.

82.   The method of claim 80 further comprising obtaining or producing the transformed or transfected cell.

10

83.   The method of claim 79 wherein the test compound comprises siRNA or shRNA comprising a nucleotide sequence set forth in any one of SEQ ID Nos: 9-140.

84.   The method of claim 79 wherein the test compound comprises antisense RNA.

15

85.   The method of claim 79 wherein the test compound comprises an antibody that binds to a BAFF protein.

86.   The method of claim 79 wherein the level of BAFF gene expression is determined
20   by a process comprising determining the level of a polypeptide encoded by the gene in the presence of the compound relative to the level of the polypeptide in the absence of the compound, wherein a reduced level of the polypeptide in the presence of the compound indicates that the compound is an antagonist of expression of the gene.

25

87.   The method of claim 86 wherein the level of the polypeptide is determined by a process comprising:
        (a)   contacting the cell or a protein extract thereof with monoclonal or polyclonal antibody that binds specifically to protein encoded by the BAFF gene under
30   conditions sufficient for an antigen-antibody complex to form; and
        (b)   detecting the antibody bound.

88.   The method of claim 87 comprising:
        (a)   providing a cell that expresses the polypeptide;
35   (b)   incubating the cell in the presence and absence of a compound to be tested;
        (c)   contacting an extract of the cell comprising the polypeptide with the antibody

under conditions sufficient for an antigen-antibody complex to form thereby capturing the polypeptide; and

(d)   detecting the antibody bound at (c).

89.   The method of claim 87 comprising:

(a)   providing a cell expresses the polypeptide;

(b)   incubating the cell in the presence and absence of a compound to be tested;

(c)   contacting an extract of the cell comprising the polypeptide with the antibody under conditions sufficient for an antigen-antibody complex to form thereby capturing the polypeptide;

(d)   contacting the captured polypeptide with an antibody that binds to the polypeptide under conditions sufficient for an antigen-antibody complex to form, wherein said antibody binds to a different epitope on the polypeptide to the antibody at (c); and

(e)   detecting the antibody bound at (d).

90.   The method of claim 79 wherein the level of BAFF gene expression is determined by a process comprising determining the level of an mRNA transcription product of the gene in the presence of the compound relative to the level of the mRNA in the absence of the compound, wherein a reduced level of the mRNA in the presence of the compound indicates that the compound is an antagonist of expression of the gene.

91.   The method of claim 90 wherein the mRNA is detected by contacting a nucleic acid probe with the mRNA transcription product in the cell or an extract thereof for a time and under conditions sufficient for hybridization to occur and then detecting the hybridization.

92.   The method of claim 91 wherein the nucleic acid probe comprises a nucleotide sequence selected from the group consisting of:

(a)   a sequence encoding an amino acid sequence having at least about 65% identity to SEQ ID NO: 2 or SEQ ID NO: 4;

(b)   a sequence having at least about 65% identity to SEQ ID NO: 1 or SEQ ID NO: 3;

(c)   a sequence that hybridizes specifically under at least low stringency conditions to SEQ ID NO: 1 or SEQ ID NO: 3;

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    (d)   a sequence comprising a protein-encoding region of (b) or (c);

    (e)   a sequence complementary to any one of (a) to (d); and

    (f)   a sequence comprising at least about 20 contiguous nucleotides of any one of (a) to (e).

93.   The method of claim 92 wherein the nucleic acid probe comprises a nucleotide sequence selected from the group consisting of SEQ ID Nos: 9-140.

94.   The method of claim 91 wherein the nucleic acid probe is labeled with a reporter molecule and hybridization is detected by detecting the reporter molecule

95.   The method of claim 91 wherein hybridization is detected by detecting nucleic acid amplified in a polymerase chain reaction (PCR).

96.   A process for identifying or determining a compound comprising:

    (a)   performing the method of claim 79 to thereby identify or determine a compound that reduces or antagonizes expression of a BAFF gene;

    (b)   optionally, determining the structure of the compound; and

    (c)   providing the compound or modulator or the name or structure of the compound.

97.   A process for producing a compound said method comprising:

    (a)   performing the method of claim 79 to thereby identify or determine a compound that reduces or antagonizes expression of a BAFF gene;

    (b)   optionally, determining the structure of the compound;

    (c)   optionally, providing the name or structure of the compound; and

    (d)   producing or synthesizing the compound.

98.   An isolated nucleic acid that antagonizes expression of a BAFF gene consisting of a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule.

99.   The isolated nucleic acid of claim 98 wherein the small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

100.   A vector comprising the isolated nucleic acid of claim 98 or 99.

101.   A cell comprising the vector of claim 100.

102.   A method of preventing or delaying the development of a cancer in a subject that
        is a candidate for anti-TNF therapy or has undergone or commenced anti-TNF
        therapy, said method comprising administering an amount of an agent that
        reduces BAFF expression to the subject sufficient to reduce the level of a BAFF
        protein or mRNA encoding a BAFF protein in a cell of the subject.

103.   The method of claim 102 wherein the agent comprises siRNA or shRNA
        comprising a nucleotide sequence selected from the group consisting of SEQ ID
        Nos: 9-140.

104.   The method of claim 102 further comprising administering an amount of an agent
        sufficient to agonize TNF or enhance the level of TNF protein or TNF activity in a
        cell of the subject.

105.   The method of claim 104 wherein the agent that agonizes TNF or enhances the
        level of TNF protein or TNF activity is co-administered with an agent that reduces
        BAFF expression in the subject such that the level of BAFF and the level of TNF
        are modulated simultaneously in the subject.

106.   The method of claim 102 wherein the cancer is a lymphoma.

107.   The method of claim 106, wherein the lymphoma is a non-Hodgkin's lymphoma
        (NHL).

108.   The method of claim 107 wherein the NHL is selected from the group consisting of
        diffuse large B cell NHL, large cell NHL, small T cell NHL, diffuse large cell NHL, B
        cell NHL, follicular mixed small and large cell NHL, mantle cell B cell NHL and
        small lymphocytic B cell NHL.

109.   The method of claim 102 wherein the cancer is a leukemia.

110.   The method of claim 102 wherein the cancer is a myeloma.

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111.   The method of claim 102 wherein the cancer is a carcinoma.

112.   The method of claim 111 wherein the carcinoma is selected from the group consisting of head and neck cancer, breast cancer, adenocarcinoma, squamous lung cancer, gastrointestinal cancer, renal cell cancer, bladder cancer, a gynecological carcinoma, prostate cancer, squamous cell carcinoma, non-squamous carcinoma, glioblastoma, epithelial vulval carcinoma and medulloblastoma.

113.   The method of claim 102 wherein the subject is a human.

114.   The method of claim 102 wherein the subject has undergone or commenced anti-TNF therapy in combination with an immunosuppressive drug.

115.   The method of claim 114 wherein the immunosuppressive drug is selected from the group consisting of methotrexate, azathioprine and a corticosteroid.

116.   The method of claim 102 wherein the subject is infected with Epstein-Barr virus (EBV).

117.   The method of claim 102 wherein the subject suffers from an autoimmune disease or inflammatory disease.

118.   The method of claim 117 wherein the autoimmune disease or inflammatory disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis and colitis.

119.   The method of claim 118 wherein the autoimmune disease or inflammatory disease is rheumatoid arthritis.

120.   The method of claim 102 wherein the subject is an aged subject.

121.   The method of claim 102 wherein the agent that reduces BAFF expression comprises a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule.

5

122.   The method of claim 121 wherein the small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

10   123.   The method of claim 102 wherein the agent that reduces BAFF expression comprises an antibody that binds to a BAFF protein thereby inhibiting BAFF activity.

124.   The method of claim 102 wherein the agent that reduces BAFF expression is
15   administered by injection.

125.   The method of claim 102 wherein the agent that reduces BAFF expression is administered by intranasal inhalation.

20   126.   Use of a composition that reduces or inhibits BAFF expression or activity in combination with an agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) for treating an inflammatory disease or autoimmune disease in a subject.

25   127.   The use according to claim 126 wherein the composition that reduces or inhibits BAFF expression comprises a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule.

128.   The use according to claim 127 wherein the small interfering RNA (siRNA) or short
30   hairpin RNA (shRNA) molecule comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

129.   The use according to claim 126 wherein the composition that reduces BAFF activity comprises an antibody that binds to a BAFF protein thereby inhibiting
35   BAFF activity.

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130.   The use according to claim 126 wherein the agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) is an antibody or polypeptide that binds to TNFα thereby reducing or inhibiting the activity of TNFα.

131.   The use according to claim 126 wherein the agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) is used in combination with an immunosuppressant drug.

132.   The use according to claim 126 wherein the immunosuppressant drug is selected from the group consisting of methotrexate, azathioprine and a corticosteroid.

133.   The use according to claim 126 wherein the inflammatory disease or autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis and colitis.

134.   The use according to claim 133 wherein the autoimmune disease or inflammatory disease is rheumatoid arthritis.

135.   Use of a composition that reduces or inhibits BAFF expression or activity in combination with an agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) in the preparation of one or more medicaments for treating an inflammatory disease or autoimmune disease in a subject.

136.   The use according to claim 135 wherein the composition that reduces or inhibits BAFF expression comprises a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule.

137.   The use according to claim 136 wherein the small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

138.   The use according to claim 135 wherein the composition that reduces BAFF activity comprises an antibody that binds to a BAFF protein thereby inhibiting BAFF activity.

5   139.   The use according to claim 135 wherein the agent that reduces or inhibits the activity of tumor necrosis factor-alpha (TNFα) is an antibody or polypeptide that binds to TNFα thereby reducing or inhibiting the activity of TNFα.

140.   The use according to claim 135 wherein the inflammatory disease or autoimmune
10   disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis and colitis.

15 141.   The use according to claim 140 wherein the autoimmune disease or inflammatory disease is rheumatoid arthritis.

142.   Use of an agent that reduces or inhibits BAFF expression or activity for treating an inflammatory disease or autoimmune disease in a subject wherein the subject is:
20   (i) a candidate for anti-TNF therapy or has undergone or commenced anti-TNF therapy; and (ii) has an enhanced BAFF activity or expression.

143.   The use according to claim 142 wherein the agent that reduces or inhibits BAFF expression comprises a small interfering RNA (siRNA) or short hairpin RNA
25   (shRNA) molecule.

144.   The use according to claim 143 wherein the small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

30
145.   The use according to claim 142 wherein the agent that reduces BAFF activity comprises an antibody that binds to a BAFF protein thereby inhibiting BAFF activity.

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146.   The use according to claim 142 wherein the anti-TNF therapy comprises administering an antibody or polypeptide that binds to TNFα thereby reducing or inhibiting the activity of TNFα.

5   147.   The use according to claim 146 wherein the anti-TNF therapy comprises administering an antibody or polypeptide that binds to TNFα thereby reducing or inhibiting the activity of TNFα in combination with an immunosuppressant drug.

148.   The use according to claim 147 wherein the immunosuppressant drug is selected
10   from the group consisting of methotrexate, azathioprine and a corticosteroid.

149.   The use according to claim 142 wherein the inflammatory disease or autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid
15   arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis and colitis.

150.   The use according to claim 149 wherein the autoimmune disease or inflammatory disease is rheumatoid arthritis.
20

151.   Use of an agent that reduces or inhibits BAFF expression or activity in the preparation of a medicament for treating an inflammatory disease or autoimmune disease in a subject wherein the subject is: (i) a candidate for anti-TNF therapy or has undergone or commenced anti-TNF therapy; and (ii) has an enhanced BAFF
25   activity or expression.

152.   The use according to claim 151 wherein the agent that reduces or inhibits BAFF expression comprises a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule.
30

153.   The use according to claim 152 wherein the small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

154. The use according to claim 151 wherein the agent that reduces BAFF activity comprises an antibody that binds to a BAFF protein thereby inhibiting BAFF activity.

5   155. The use according to claim 151 wherein the anti-TNF therapy comprises administering an antibody or polypeptide that binds to TNFα thereby reducing or inhibiting the activity of TNFα.

156. The use according to claim 155 wherein the anti-TNF therapy comprises
10   administering an antibody or polypeptide that binds to TNFα thereby reducing or inhibiting the activity of TNFα in combination with an immunosuppressant drug.

157. The use according to claim 156 wherein the immunosuppressant drug is selected from the group consisting of methotrexate, azathioprine and a corticosteroid.

15

158. The use according to claim 151 wherein the inflammatory disease or autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis,
20   psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis and colitis.

159. The use according to claim 158 wherein the autoimmune disease or inflammatory disease is rheumatoid arthritis.

25   160. A commercial package comprising:
   (a)   a composition that reduces or inhibits BAFF expression or activity in a subject;
   (b)   an agent that reduces or inhibits TNFα activity; and
   (c)   instructions for use of the composition and agent for treating an
30   inflammatory disease or autoimmune disease in a subject.

161. The commercial package according to claim 160 wherein the composition that reduces or inhibits BAFF expression comprises a small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule.

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162.   The commercial package according to claim 161 wherein the small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecule comprises a nucleotide sequence selected from the group set forth in SEQ ID Nos: 9-140.

163.   The commercial package according to claim 160 wherein the composition that reduces BAFF activity comprises an antibody that binds to a BAFF protein thereby inhibiting BAFF activity.

164.   The commercial package according to claim 160 wherein the agent that reduces or inhibits TNFα comprises an antibody or polypeptide that binds to TNFα thereby reducing or inhibiting the activity of TNFα.

165.   The commercial package according to claim 160 wherein the inflammatory disease or autoimmune disease is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Crohn's Disease, Crohn's disease in which fistulas form, rheumatoid arthritis, juvenile rheumatoid arthritis, active ankylosing spondylitis, psoriasis, psoriatic arthritis, reactive arthritis, inflammatory bowel disease arthritis and colitis.

166.   The commercial package according to claim 165 wherein the autoimmune disease or inflammatory disease is rheumatoid arthritis.
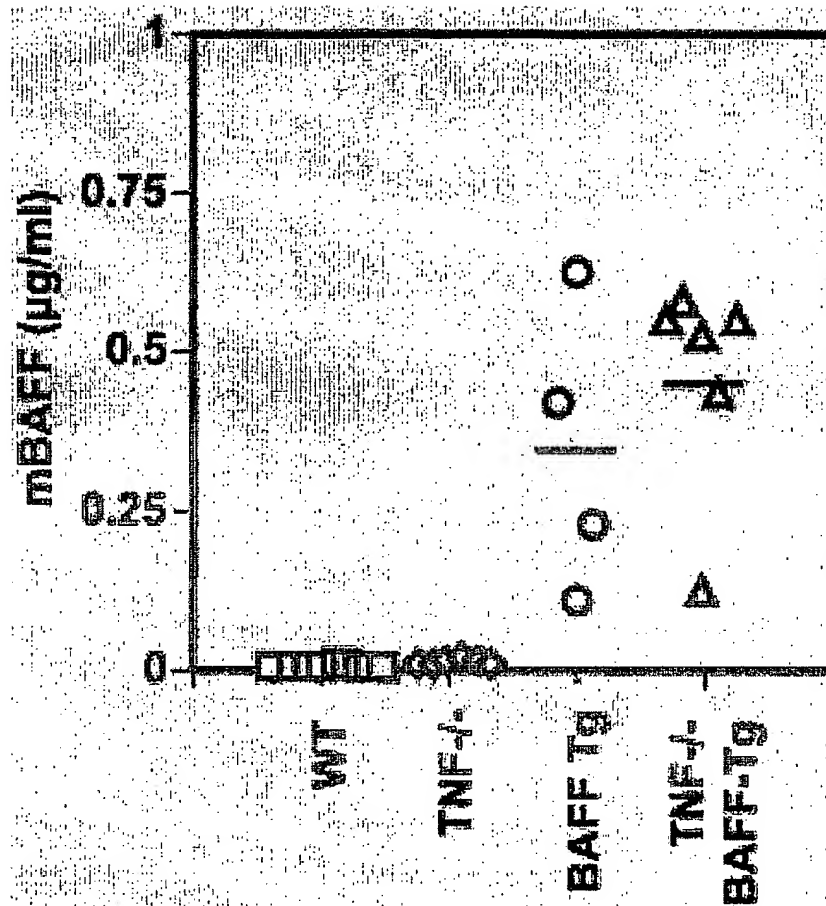
FIGURE 1a

FIGURE 1b

FIGURE 1c

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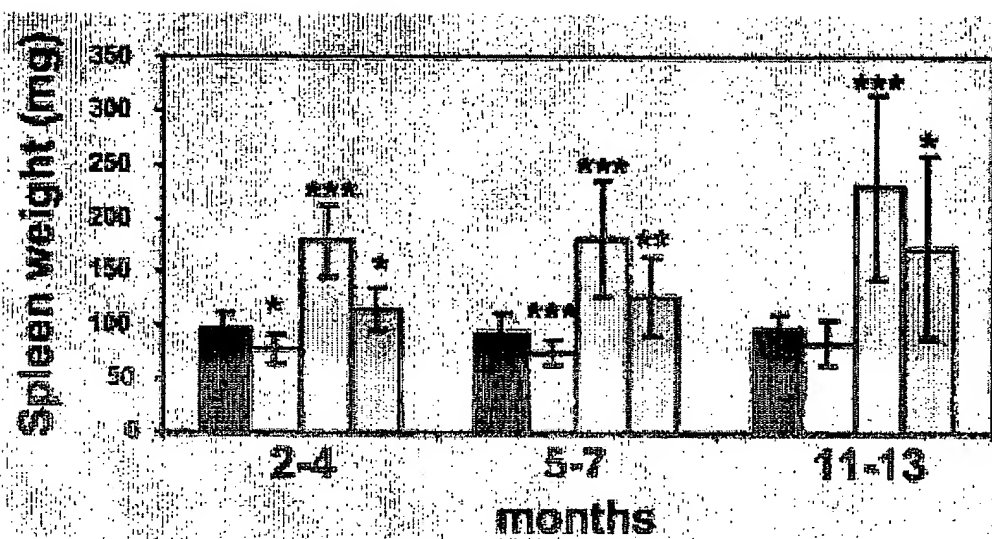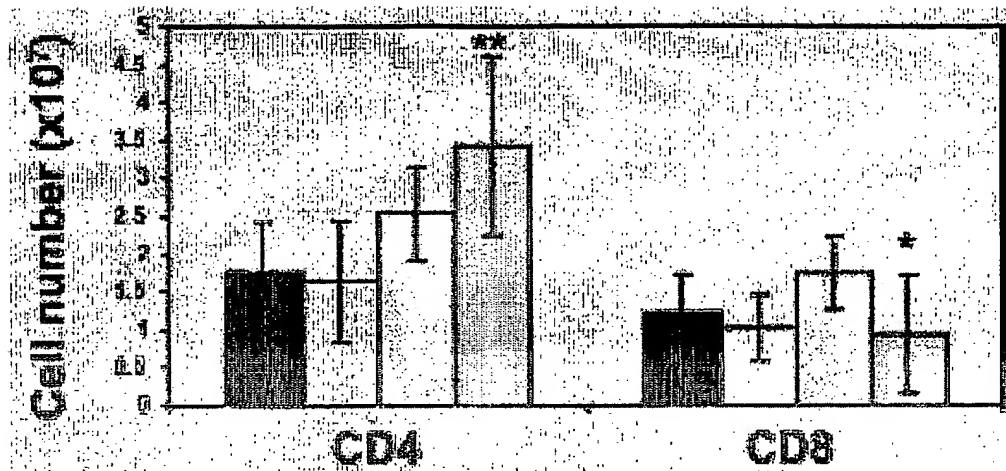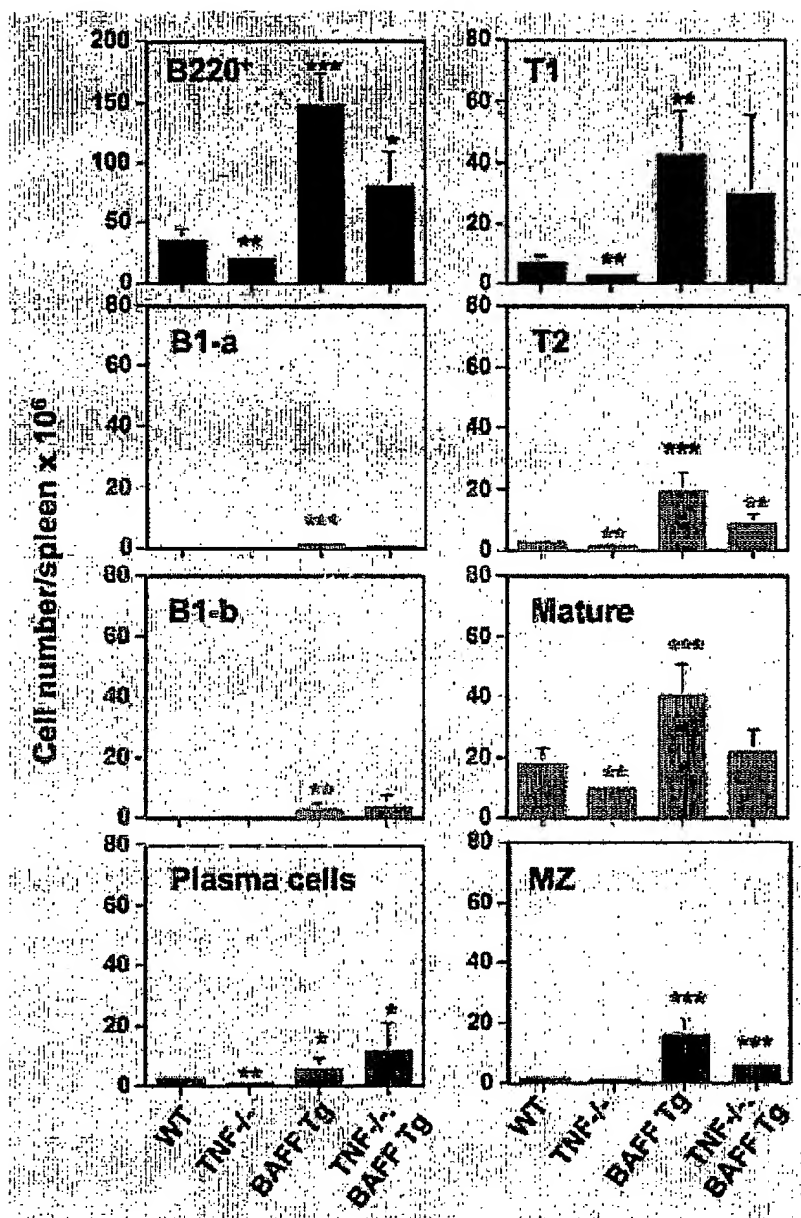
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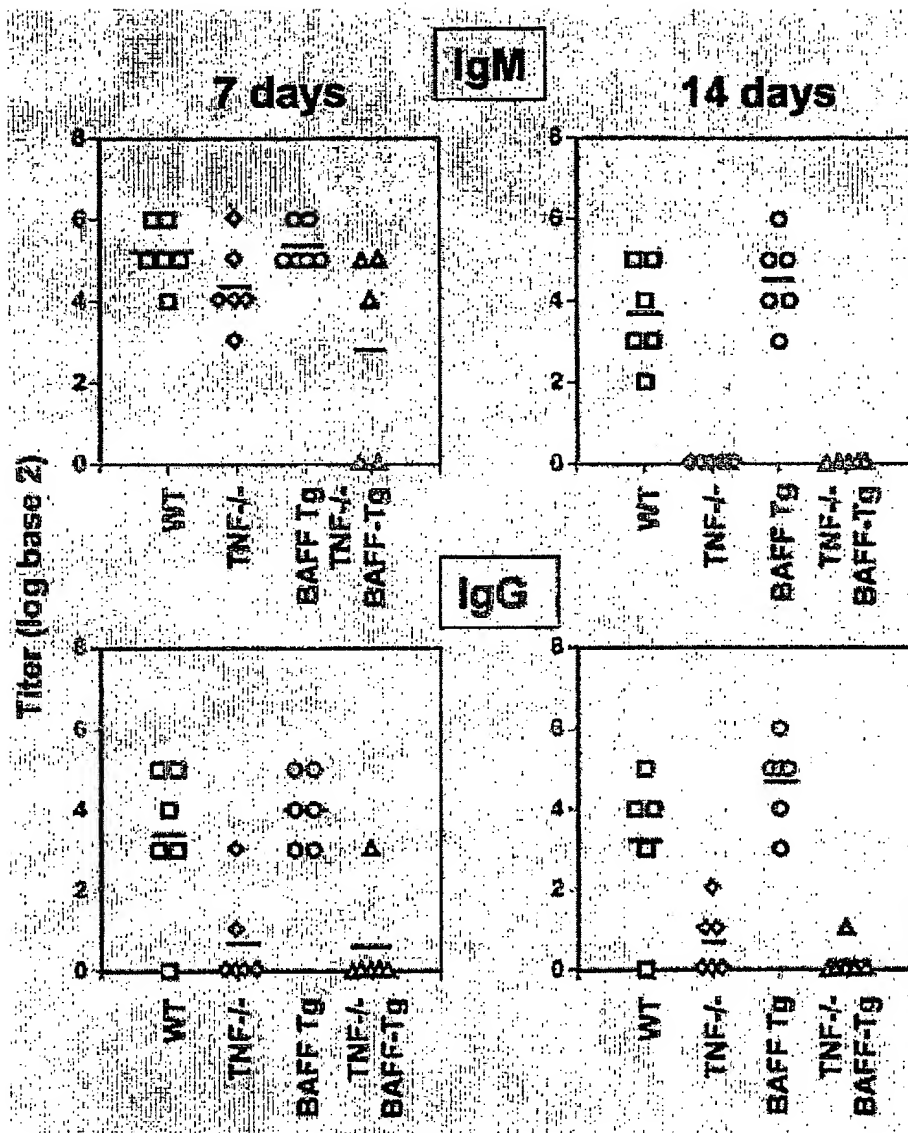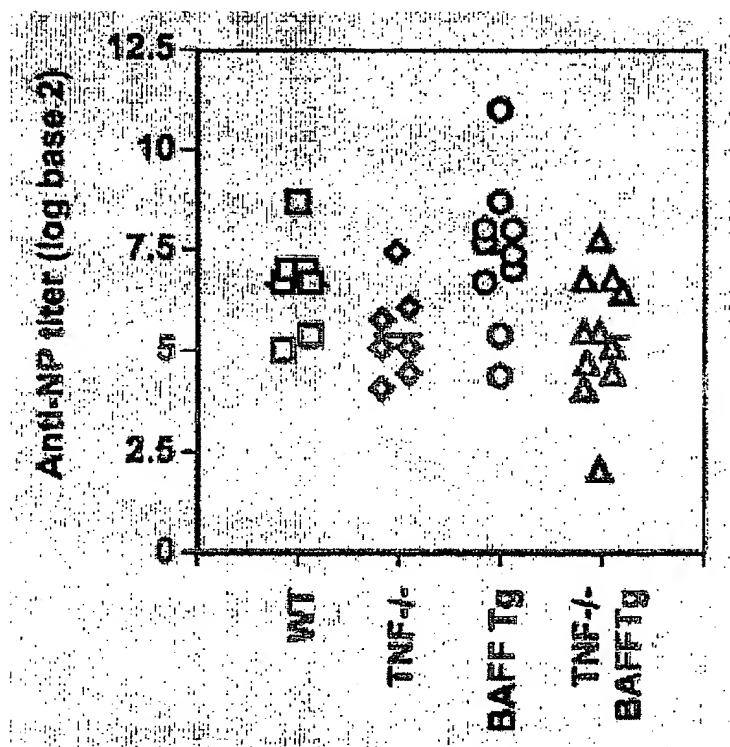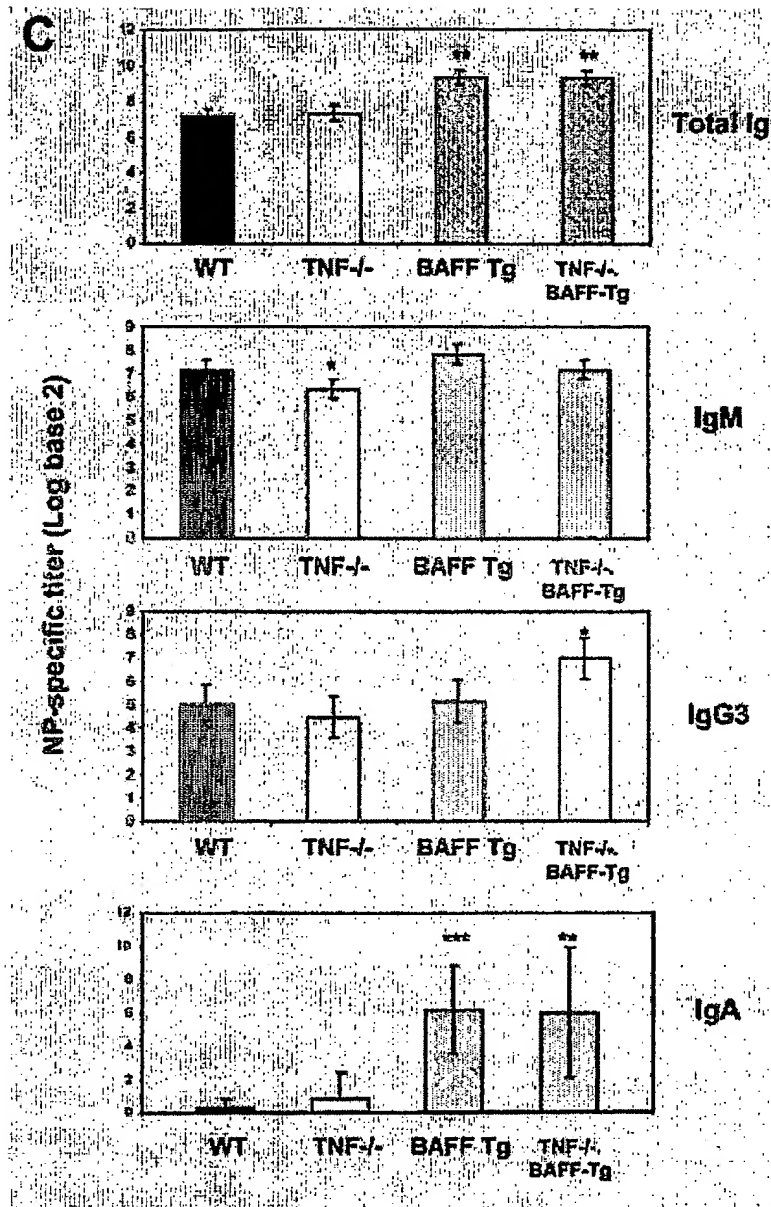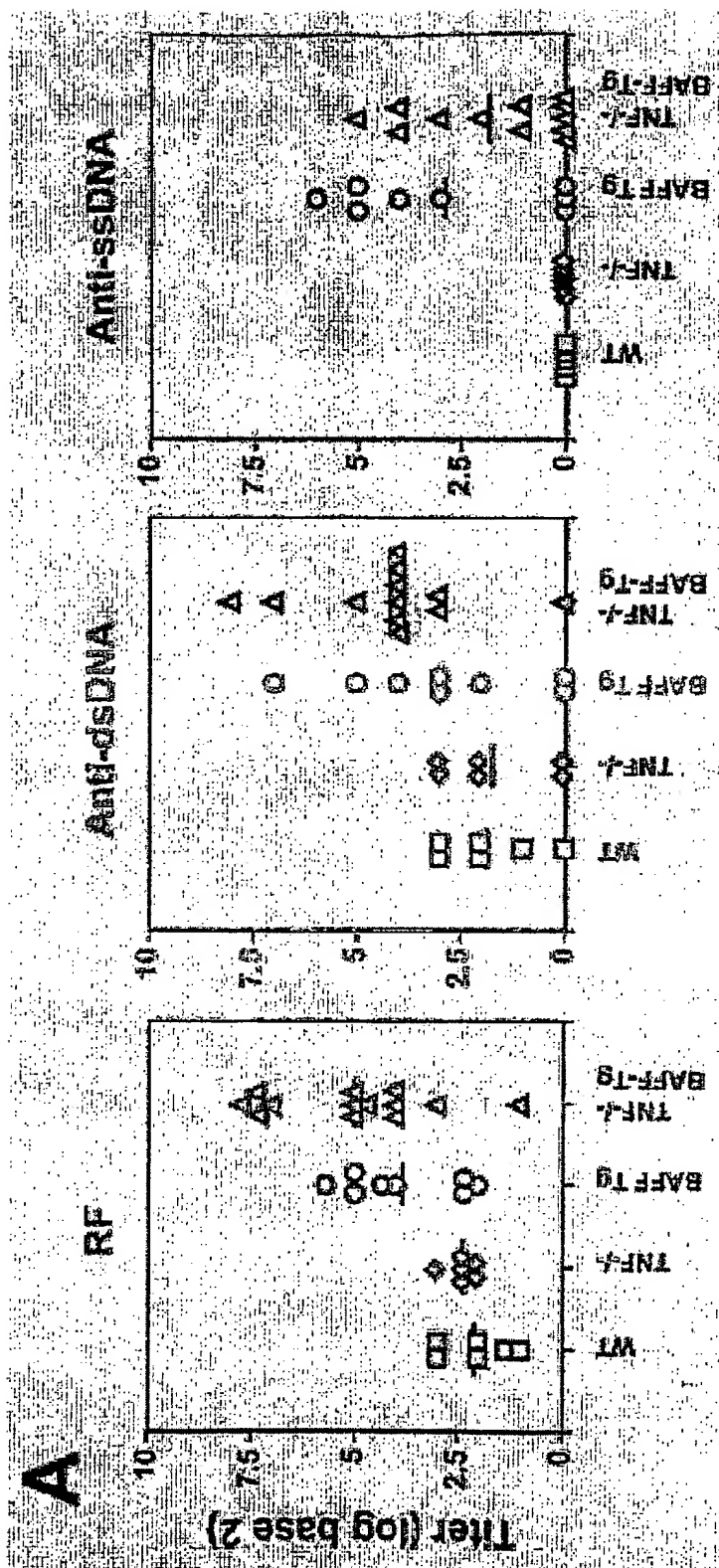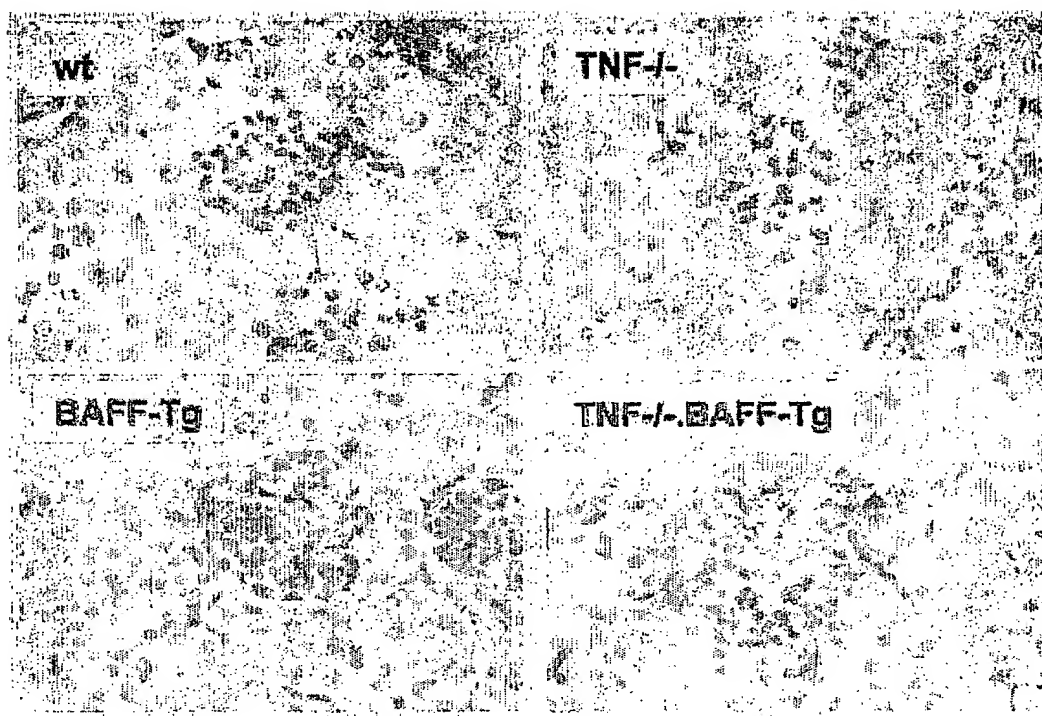
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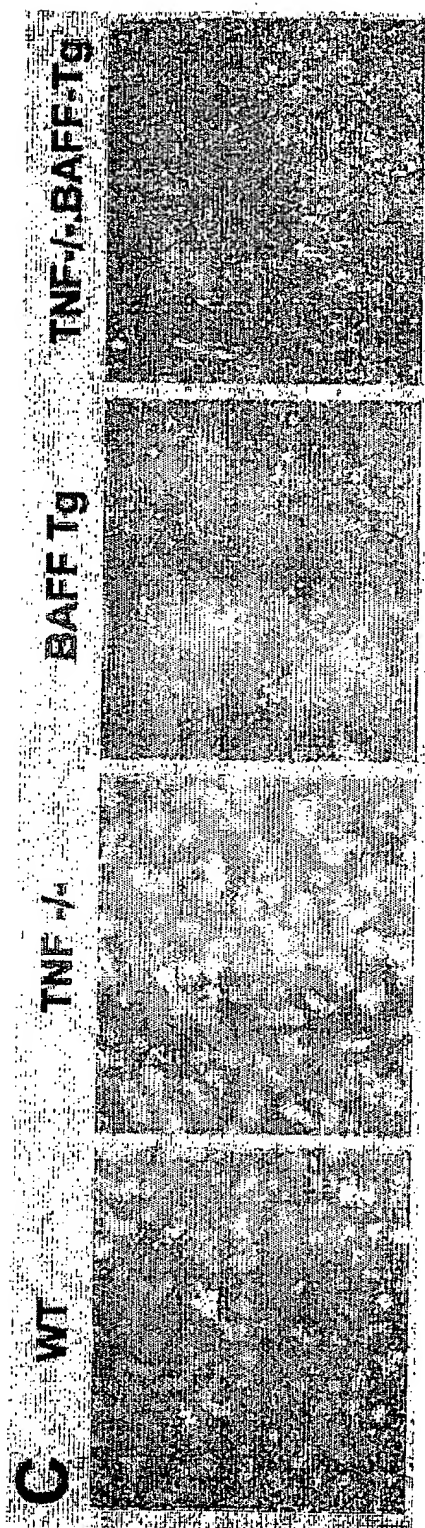
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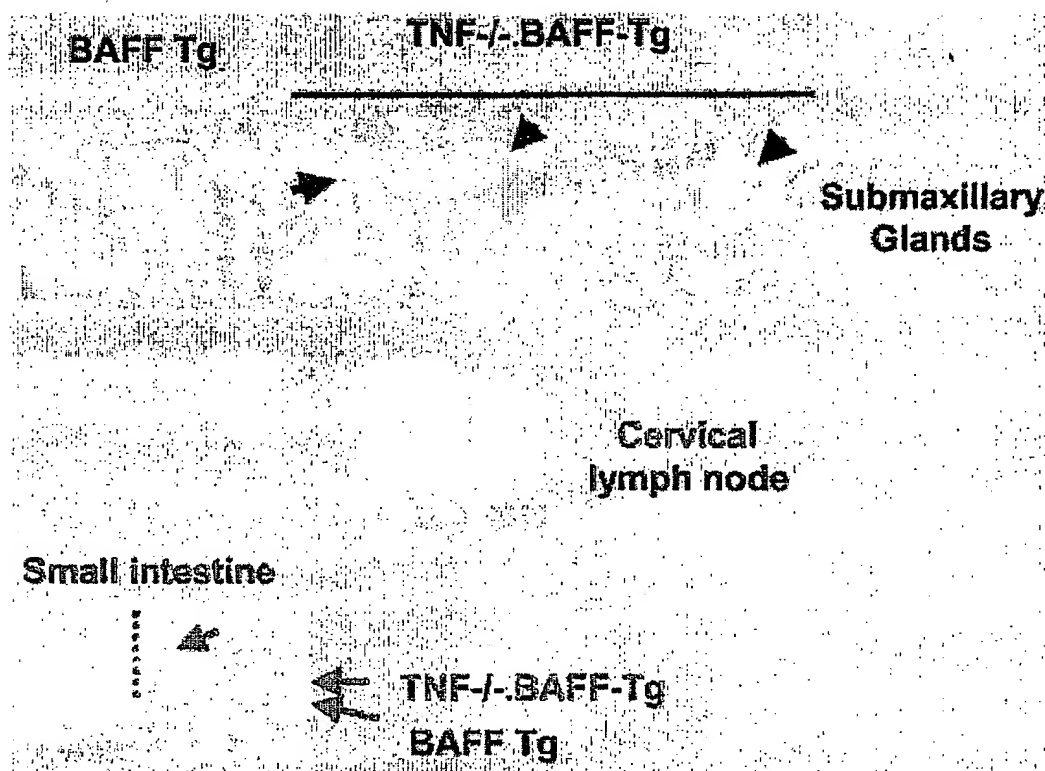
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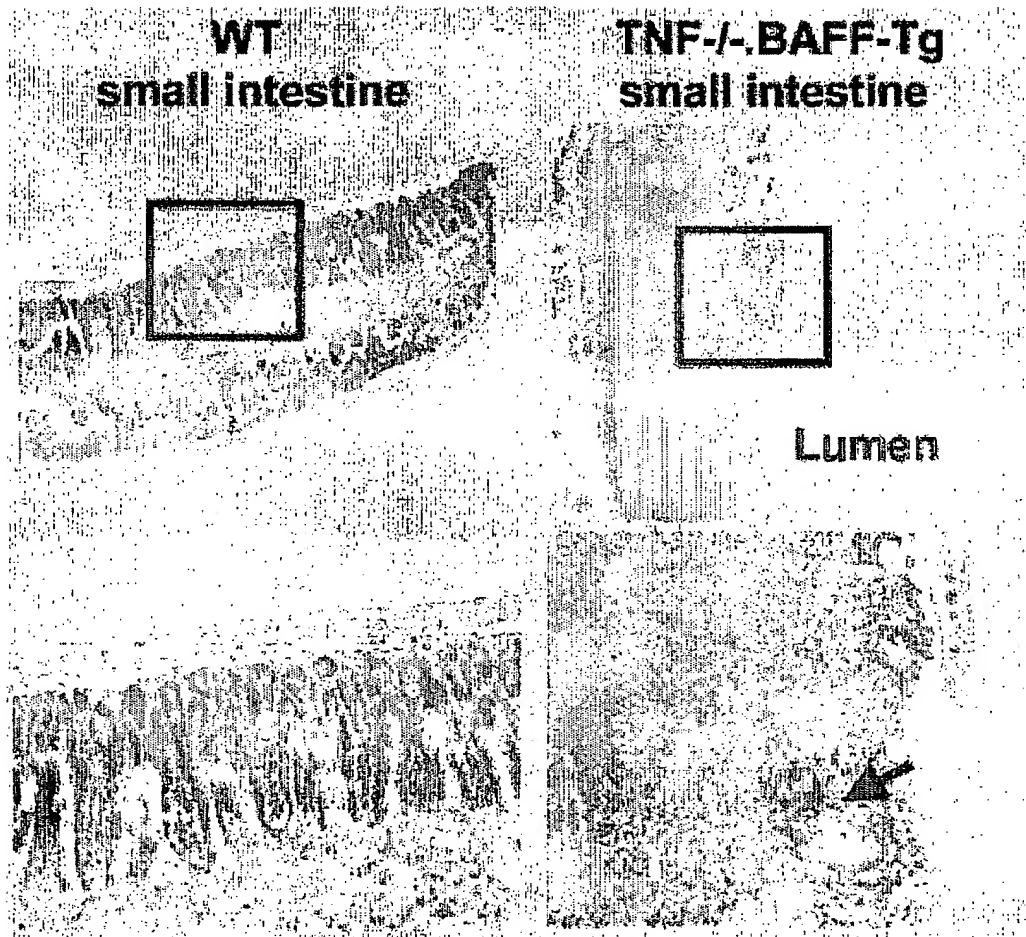
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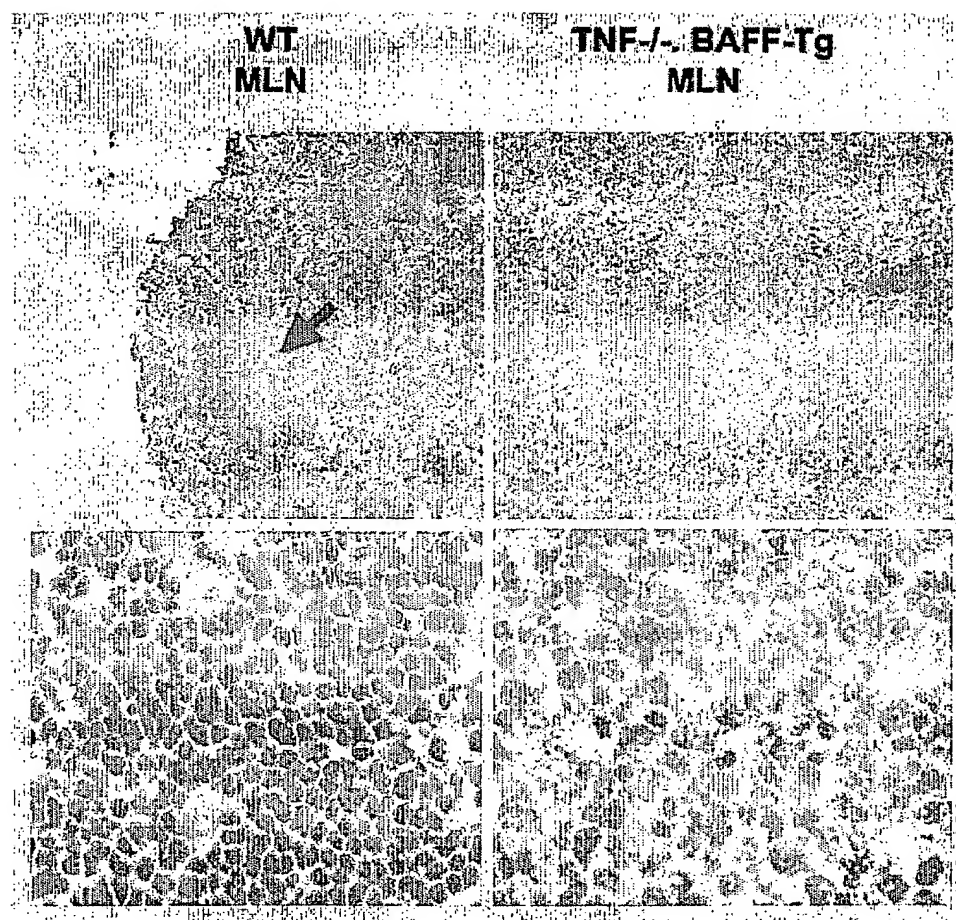
FIGURE 4a

FIGURE 4b

FIGURE 4c

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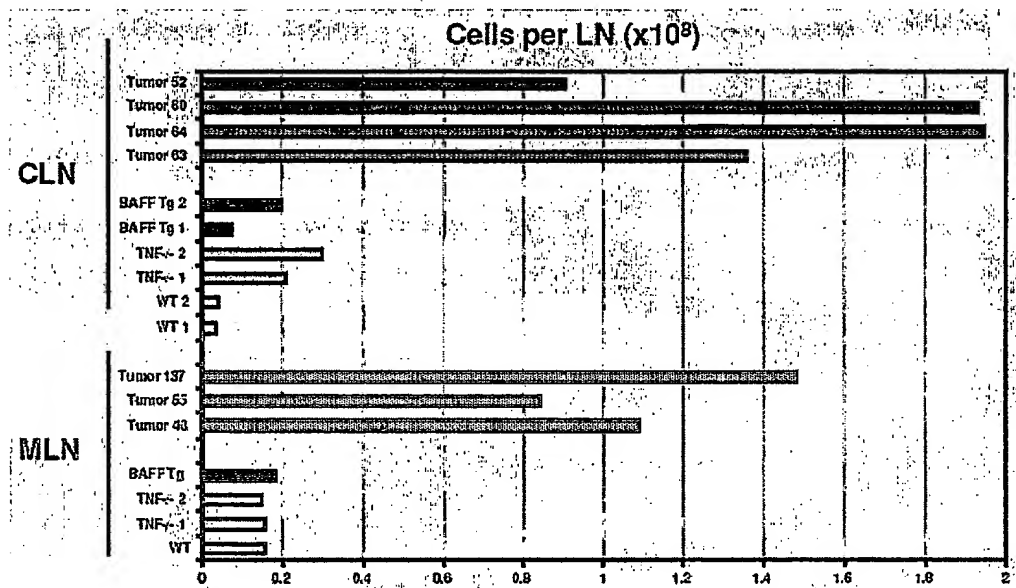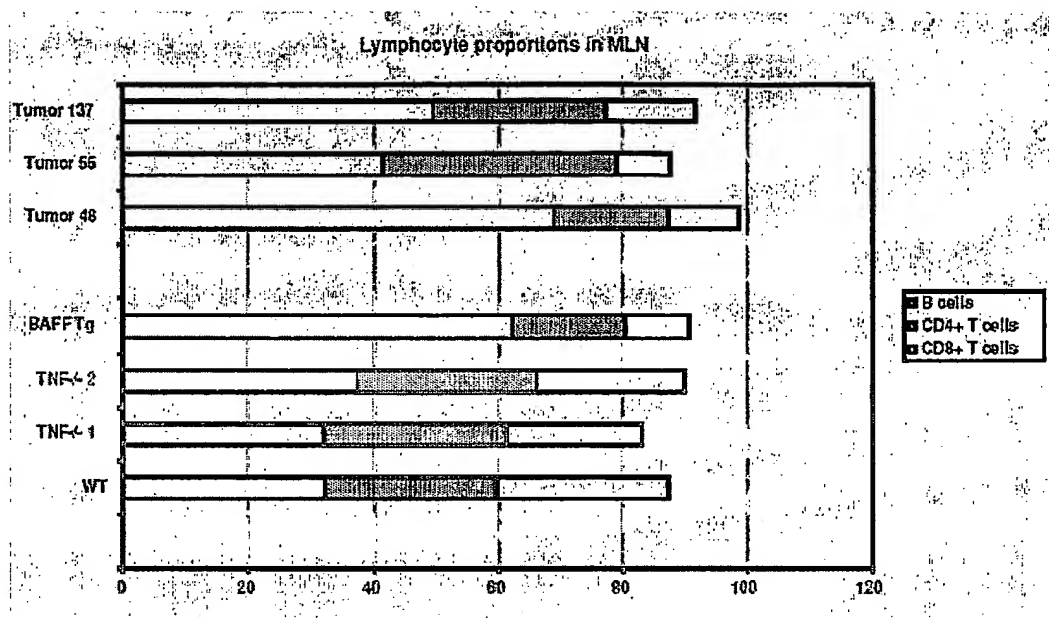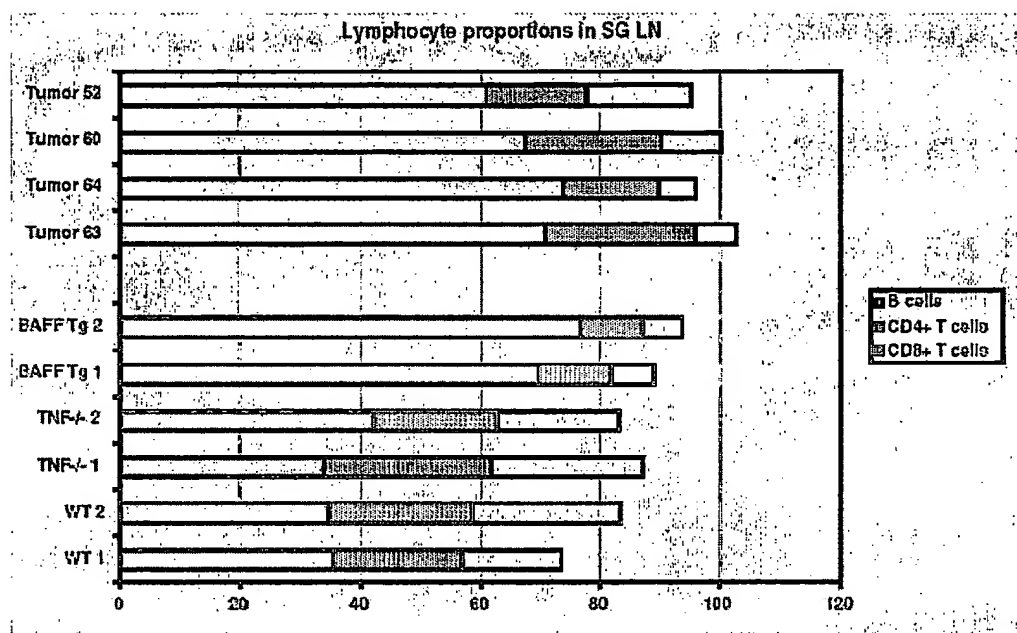
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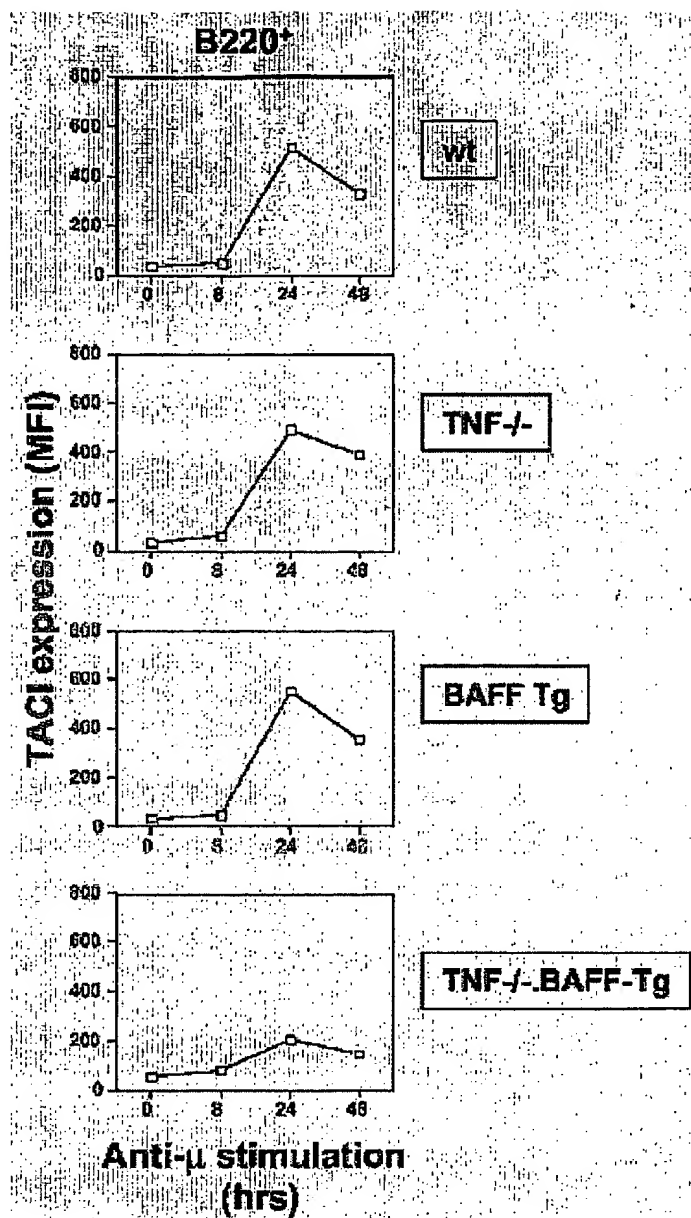
FIGURE 6

FIGURE 7

FIGURE 8

-1-

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    Pro Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro Gly
                    110                 115                 120

5   gaa ggc aac tcc agt cag aac agc aga aat aag cgt gcc gtt cag ggt      678
    Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val Gln Gly
                    125                 130                 135

    cca gaa gaa aca gtc act caa gac tgc ttg caa ctg att gca gac agt      726
10  Pro Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser
                    140                 145                 150

    gaa aca cca act ata caa aaa gga tct tac aca ttt gtt cca tgg ctt      774
    Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe Val Pro Trp Leu
15      155                 160                 165

    ctc agc ttt aaa agg gga agt gcc cta gaa gaa aaa gag aat aaa ata      822
    Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile
    170                 175                 180                 185
20
    ttg gtc aaa gaa act ggt tac ttt ttt ata tat ggt cag gtt tta tat      870
    Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr
                    190                 195                 200

25  act gat aag acc tac gcc atg gga cat cta att cag agg aag aag gtc      918
    Thr Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val
                    205                 210                 215

    cat gtc ttt ggg gat gaa ttg agt ctg gtg act ttg ttt cga tgt att      966
30  His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile
                    220                 225                 230

    caa aat atg cct gaa aca cta ccc aat aat tcc tgc tat tca gct ggc     1014
    Gln Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly
35      235                 240                 245

    att gca aaa ctg gaa gaa gga gat gaa ctc caa ctt gca ata cca aga     1062
    Ile Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg
    250                 255                 260                 265
40
    gaa aat gca caa ata tca ctg gat gga gat gtc aca ttt ttt ggt gca     1110
    Glu Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala
                    270                 275                 280

45  ttg aaa ctg ctg tgacctactt acaccatgtc tgtagctatt ttcctccctt         1162
    Leu Lys Leu Leu
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    tctctgtacc tctaagaaga aagaatctaa ctgaaaatac ca                      1204
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        Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
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        Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
                50                      55                      60

        Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
10      65                      70                      75                      80

        Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
                        85                      90                      95

15      Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
                    100                     105                     110

        Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
                    115                     120                     125
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        Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln
                130                     135                     140

        Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys
25      145                     150                     155                     160

        Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
                        165                     170                     175

30      Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
                    180                     185                     190

        Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
                    195                     200                     205
35
        Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
                210                     215                     220

        Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
40      225                     230                     235                     240

        Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
                        245                     250                     255

45      Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
                    260                     265                     270

        Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
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       tgctctgagg gcagtctccc aggacacaga tgacaggaaa tgacccaccc ctgtggtcac     180

       ttactccaaa ggcctagacc ttcaaagtgc tcctcgtgga atg gat gag tct gca      235
10                                                   Met Asp Glu Ser Ala
                                                     1               5

       aag acc ctg cca cca ccg tgc ctc tgt ttt tgc tcc gag aaa gga gaa      283
       Lys Thr Leu Pro Pro Pro Cys Leu Cys Phe Cys Ser Glu Lys Gly Glu
15                      10              15                  20

       gat atg aaa gtg gga tat gat ccc atc act ccg cag aag gag gag ggt      331
       Asp Met Lys Val Gly Tyr Asp Pro Ile Thr Pro Gln Lys Glu Glu Gly
20                  25              30                  35

       gcc tgg ttt ggg atc tgc agg gat gga agg ctg ctg gct gct acc ctc      379
       Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu Leu Ala Ala Thr Leu
                    40              45                  50

       ctg ctg gcc ctg ttg tcc agc agt ttc aca gcg atg tcc ttg tac cag      427
25     Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala Met Ser Leu Tyr Gln
                55              60                  65

       ttg gct gcc ttg caa gca gac ctg atg agc ctg cgc atg gag ctg cag      475
       Leu Ala Ala Leu Gln Ala Asp Leu Met Ser Leu Arg Met Glu Leu Gln
30     70                  75                  80                  85

       agc tac cga ggt tca gca aca cca gcc gcc gcg ggt gct cca gag ttg      523
       Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala Gly Ala Pro Glu Leu
                    90              95                  100
35
       acc gct gga gtc aaa ctc ctg aca ccg gca gct cct cga ccc cac aac      571
       Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala Pro Arg Pro His Asn
                    105             110                 115

40     tcc agc cgc ggc cac agg aac aga cgc gct ttc cag gga cca gag gaa      619
       Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe Gln Gly Pro Glu Glu
                120             125                 130

       aca gaa caa gat gta gac ctc tca gct cct cct gca cca tgc ctg cct      667
45     Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro Ala Pro Cys Leu Pro
                135             140                 145

       gga tgc cgc cat tct caa cat gat gat aat gga atg aac ctc aga aac      715
       Gly Cys Arg His Ser Gln His Asp Asp Asn Gly Met Asn Leu Arg Asn
50     150             155                 160                 165

       atc att caa gac tgt ctg cag ctg att gca gac agc gac acg ccg act      763
       Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp Ser Asp Thr Pro Thr
                    170             175                 180
55
       ata cga aaa gga act tac aca ttt gtt cca tgg ctt ctc agc ttt aaa      811
       Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys
                    185             190                 195

60     aga gga aat gcc tta gag gag aaa gag aac aaa ata gtg gtg agg caa      859
       Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys Ile Val Val Arg Gln
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            200                 205                 210

     aca ggc tat ttc ttc atc tac agc cag gtt cta tac acg gac ccc atc     907
     Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu Tyr Thr Asp Pro Ile
  5      215             220             225

     ttt gct atg ggt cat gtc atc cag agg aag aaa gta cac gtc ttt ggg     955
     Phe Ala Met Gly His Val Ile Gln Arg Lys Lys Val His Val Phe Gly
 10  230             235             240             245

     gac gag ctg agc ctg gtg acc ctg ttc cga tgt att cag aat atg ccc    1003
     Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro
                 250             255             260

 15  aaa aca ctg ccc aac aat tcc tgc tac tcg gcc ggc atc gcg agg ctg    1051
     Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Arg Leu
                 265             270             275

     gaa gaa gga gat gag att cag ctt gca att cct cgg gag aat gca cag    1099
     Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln
 20          280             285             290

     att tca cgc aac gga gac gac acc ttc ttt ggt gcc cta aaa ctg ctg    1147
     Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly Ala Leu Lys Leu Leu
 25      295             300             305

     taactcactt gctggagtgc gtgaccccct tccctcgtct tctctgtacc tccgagggag   1207

     aaacagacga ctggaaaaac taaagatggg ggaaagccgt cagcgaaagt tttctcgtga   1267
 30
     cccgttgaat ctgatccaaa ccaggaaata taacagacag ccacaaccga agtgtgccat   1327

     gtgagttatg agaaacggag cccgcgctca gaaagaccag atgaggaagg ccgttttctc   1387

 35  cagtcctttg ccaacacgca ccgcaacctt gcttttttgcc ttgggtgaca catgttcaga   1447

     atgcagggag atttccttgt tttgcgattt gccatgagaa gagggcccac aactgcaggt   1507

     cactgaagca ttcacgctaa gtctcaggat ttactctccc ttctcatgct aagtacacac   1567
 40
     acgctctttt ccaggtaata ctatgggata ctatggaaag gttgtttgtt tttaaatcta   1627

     gaagtcttga actggcaata gacaaaaatc cttataaatt caagtgtaaa ataaacttaa   1687

 45  ttaaaaaggt ttaagtgtga aaaaaaa                                        1714
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                20                  25                  30

 60 Gln Lys Glu Glu Gly Ala Trp Phe Gly Ile Cys Arg Asp Gly Arg Leu
            35                  40                  45
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     Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Ser Ser Phe Thr Ala
         50              55              60

5    Met Ser Leu Tyr Gln Leu Ala Ala Leu Gln Ala Asp Leu Met Ser Leu
     65              70              75              80

     Arg Met Glu Leu Gln Ser Tyr Arg Gly Ser Ala Thr Pro Ala Ala Ala
                 85              90              95
10
     Gly Ala Pro Glu Leu Thr Ala Gly Val Lys Leu Leu Thr Pro Ala Ala
                 100             105             110

     Pro Arg Pro His Asn Ser Ser Arg Gly His Arg Asn Arg Arg Ala Phe
15          115             120             125

     Gln Gly Pro Glu Glu Thr Glu Gln Asp Val Asp Leu Ser Ala Pro Pro
         130             135             140

20   Ala Pro Cys Leu Pro Gly Cys Arg His Ser Gln His Asp Asp Asn Gly
     145             150             155             160

     Met Asn Leu Arg Asn Ile Ile Gln Asp Cys Leu Gln Leu Ile Ala Asp
                 165             170             175
25
     Ser Asp Thr Pro Thr Ile Arg Lys Gly Thr Tyr Thr Phe Val Pro Trp
                 180             185             190

     Leu Leu Ser Phe Lys Arg Gly Asn Ala Leu Glu Glu Lys Glu Asn Lys
30           195             200             205

     Ile Val Val Arg Gln Thr Gly Tyr Phe Phe Ile Tyr Ser Gln Val Leu
         210             215             220

35   Tyr Thr Asp Pro Ile Phe Ala Met Gly His Val Ile Gln Arg Lys Lys
     225             230             235             240

     Val His Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys
                 245             250             255
40
     Ile Gln Asn Met Pro Lys Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala
                 260             265             270

     Gly Ile Ala Arg Leu Glu Glu Gly Asp Glu Ile Gln Leu Ala Ile Pro
45           275             280             285

     Arg Glu Asn Ala Gln Ile Ser Arg Asn Gly Asp Asp Thr Phe Phe Gly
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    <213>  TNF- specific primer antisense-L
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  60  <210>  21
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- 16 -

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	INTERNATIONAL SEARCH REPORT	International application No.
		PCT/AU2004/000215

A.	CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12Q 1/68; G01N 33/53; C12N 15/19; C07K 14/52, 16/18; A61K 48/00, 39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, Medline, CA. Baff/Tall-1/Thank/BLys/zTNF4, antagonist/inhibitor, antisense/RNAi/ribozyme, autoimmune disease, gene/protein expression, cancer/tumor/tumour/lymphoma/melanoma/leukaemia/leukemia/neoplasm.

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	Batten, Marcel et al. TNF deficiency fails to protect BAFF transgenic mice against autoimmunity and reveals a predisposition to B cell lymphoma. J Immunol, 2004. 172: 812-822. Whole document.	All
Y	Reimold AM. TNFα as therapeutic target: new drugs, more applications. Curr Drug Targets Inflamm Allergy, 2002. 1(4): 377-92. Review. Whole document.	1-78, 102-159,
Y	Brown SL et al. Tumor necrosis factor antagonist therapy and lymphoma development: twenty-six cases reported to the Food and Drug Administration. Arthritis Rheum, 2002. 46(12): 3151-8. Whole document.	1-78, 102-159, 126-166

[X] Further documents are listed in the continuation of Box C   [X] See patent family annex

*	Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
14 May 2004	**2 0 MAY 2004**

Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**Gillian Allen** Telephone No : (02) 6283 2266

Form PCT/ISA/210 (second sheet) (January 2004)

C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Mackay Fabienne et al. BAFF: a fundamental survival factor for B cells. Nature Reviews/ Immunonlogy, 2002. 2:465-75. Whole document, particularly Box 1 BAFF and cancer, p473.	1-78, 102-159, 126-166
Y	Seshasayee Dhaya et al. Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. Immunity, 2003 Feb 18. (2) 279-88. Particularly Results and Discussion.	1-78, 102-159, 126-166
Y	WO 2001/06037 A1 (GENENTECH, INC). 23 August 2001. Whole document, particularly claims 46-52	1-78, 102-159, 126-166
Y	WO 2002/016411 A2 (HUMAN GENOME SCIENCES, INC). 28 February 2002. Whole document, particularly claims 1-15, 29, 44-54.	1-78, 102-159, 126-166
Y	WO 2001/012812 A2 (BIOGEN, INC). 22 February 2001. Claims 4, 7, 17	1-78, 102-159, 126-166
Y	Nardelli Bernardetta et al. B lymphocyte stimulator(BLyS): a therapeutic trichotomy for the treatment of B lymphocyte diseases. Leukemia and Lymphoma, 2002. 43 (7) 1367-73. Whole document.	1-78, 102-159, 126-166
Y	Mackay Fabienne et al. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. J Exp Med, 1999. 190(11): 1697-1710. Whole document.	1-78, 102-159, 126-166
Y	Kalled SL et al. BAFF: B cell survival factor and emerging therapeutic target for autoimmune disorders. Expert Opin Ther Targets, Feb 2003. 7(1): 115-23. Whole document.	126-166
X	US 2002/0037852 A2 (BROWNING, Jeffrey et al) 28 March 2002. P8, col 2, paras 0063 and 0064.	79-101
Y		126-166

| Box No. II | Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet) |

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:

    because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos

    because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

    because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

| Box No. III | Observations where unity of invention is lacking (Continuation of item 3 of first sheet) |

This International Searching Authority found multiple inventions in this international application, as follows:

    The ISA found 4 inventions, see Supplemental Box III for details

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☒ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Supplemental Box III
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: III

Lack of Unity

The ISA has identified four inventions.

- Invention no 1, defined by claims 1-78 and 102-125, is directed towards diagnosis and treatment of cancer in persons undergoing or proposed to be undergoing anti-TNF-therapy, wherein diagnosis is based on BAFF expression levels, and treatment is by BAFF antagonists.

- Invention no 2, defined by claims 79-97, is directed to methods of identifying compounds, particularly antisense constructs, that reduce or antagonise expression of the BAFF gene or protein..

- Invention no 3 defined by claims 98-101 and 160-166 is to BAFF antagonists *per se*, specifically those controlling BAFF gene expression.

- Invention no 4, defined by claims 126-159 is directed to treating autoimmune or inflammatory disease with BAFF antagonists and anti-TNF therapy.

BAFF is a known protein (page 9, line 36 - p 10, line 34 of the present application).

The only common feature between inventions 1-4 is BAFF antagonists, and these are known (see abstract of WO 2001/06397, attached), which also discloses treatment of autoimmune disease and cancer with BAFF antagonists. Tall-1 is an alternative name for BAFF, and TACI and BCMA are BAFF receptors, whose interaction with BAFF induces the signal transduction cascades that provide "BAFF activity".

Invention 4 is to treatment of autoimmune disease with two substances, one having anti-TNF activity, and one having anti-BAFF activity, both of which are known to have therapeutic activity against such disease. Anti-TNF therapy is disclosed as a standard method of the art by the present applicants, and treatment of autoimmune diseases with BAFF antagonists is disclosed by WO 2001/06397, for example. Combination therapies cannot be considered inventive in the absence of any disclosure of unexpected effects or synergistic action between the two forms of treatment. Thus combination therapy with BAFF antagonists and anti-TNF cannot be accepted as a special technical feature that unites inventions 1 and 4

There is therefore no special technical feature that unites the four inventions.

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
WO 2002/016411	AU 200188301
	US 2003091565
WO 2001/060397	AU 200120485
	CA 2396793
	EP 1255558
	JP 2003522800
WO 2001/012812	AU 200069112
	CA 2383154
	CN 1379815
	EP 1210425
	JP 2003507364
	US 2002165156
	US 2002172674
US 2002037852	CA 2360062
	EP 1146892

END OF ANNEX